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4 "The green peach aphid, *Myzus persicae,* transcriptome in response to a circulative,
5 nonpropagative polerovirus, *Potato leafroll virus*".
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21 **Abstract:**

22 Viruses in the *Luteoviridae* family, such as *Potato leafroll virus* (PLRV), are transmitted by
23 aphids in a circulative and nonpropagative mode. This means the virions enter the aphid body
24 through the gut when they feed from infected plants and then the virions circulate through the
25 hemolymph to enter the salivary glands before being released into the saliva. Although these
26 viruses do not replicate in their insect vectors, previous studies have demonstrated viruliferous
27 aphid behavior is altered and the obligate symbiont of aphids, *Buchnera aphidocola,* may be
28 involved in transmission. Here we provide the transcriptome of green peach aphids (*Myzus*
29 *persicae*) carrying *PLRV* and virus-free control aphids using Illumina sequencing. Over 150
30 million paired-end reads were obtained through Illumina sequencing, with an average of 19
31 million reads per library. The comparative analysis identified 134 differentially expressed genes
32 (DEGs) between the *M. persicae* transcriptomes, including 64 and 70 genes that were down- and
33 up-regulated in aphids carrying PLRV, respectively. Using functional classification in the GO
34 databases, 80 of the DEGs were assigned to 391 functional subcategories at category level 2. The
35 most highly up-regulated genes in aphids carrying PLRV were cytochrome p450s, genes related
36 to cuticle production, and genes related to development, while genes related to histone and
37 histone modification were the most down-regulated. PLRV aphids had reduced *Buchnera* titer
38 and lower abundance of several *Buchnera* transcripts related to stress responses and metabolism.
39 These results suggest carrying PLRV may reduce both aphid and *Buchnera* genes in response to
40 stress. This work provides valuable basis for further investigation into the complicated
41 mechanisms of circulative and nonpropogative transmission.

42

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43 **Introduction:**

44 Aphids are a member of the superfamily Aphidoidea, are distributed world-wide, and cause
45 major damage to global agricultural (1). Despite there being over 4,000 species, only about 400
46 are known as significant pests (2). Aphids are effective pests partially because they do not
47 require sexual reproduction and can use parthenogenesis to quickly increase their numbers (3).
48 Another aspect of aphid biology that makes them an effective pest is their host range. Although
49 many aphids are very specialized herbivores, only feeding on a few related species, some species
50 feed on many taxa of plants (4). *Myzus persicae* is one of these polyphagous pests, feeding on
51 over 40 different families, including Solanaceae (5).

52 Along with causing direct feeding damage, aphids are important plant virus vectors,
53 representing over 50% of all known insect vectors for plant viruses (6)(7,8). Increasing evidence
54 has shown that plant viruses alter vector host finding, dispersal, and inoculation through changes
55 in host physiology, however the underlying mechanisms are largely unknown (9,10)(11). Recent
56 evidence suggests that viruses may also directly affect aphid biology (12–15). *Rhopalosiphum*
57 *padi* aphids carrying *Barley yellow dwarf virus* (BYDV), a virus from the genus *Luteovirus*,
58 prefer non-infected hosts, while virus free *R. padi* prefer infected hosts (12). In these
59 experiments, the authors determined this interaction was due to direct impacts on the aphid and
60 not due to virus induced changes in host plant quality by purifying BYDV virions and feeding
61 them to aphids in artificial diet before conducting aphid bioassays.

62 Although their lifestyle or host may change, all aphids depend on *Buchnera aphidicola* as
63 their primary obligate endosymbiont (16). *Buchnera* provide the aphid with essential amino
64 acids and nutrients that are limited in the aphid's diet (17–20), and because of this aphids can no
65 longer survive without *Buchnera.* For example, when *Buchnera* is reduced by using antibiotics,

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66 studies have shown lower body mass, lower fecundity, and changes to feeding behavior (21,22).
67 Essential components of the *Buchnera* genome have been lost (23,24) as *Buchnera* co-diversified
68 with aphids over time (16,25,26). Because of this *Buchnera* also depends on aphids for survival,
69 living inside special aphid cells, known as "bacteriocytes". Together, however, this mutualistic
70 duo wreaks havoc on agriculture across the globe.

71 Previous studies have speculated *Buchnera* may have a role in aphid transmission of
72 plant viruses (26–30). Specifically, the *Buchnera* chaperone protein GroEL, a homologue from
73 *Escherichia coli* (31), has been implicated in transmission for a number of viruses (26,27,30,32–
74 35). Direct interactions are thought to be unlikely due to the spatial separation of bacteriocytes
75 and circulating virions (26). However, GroEL from *Buchnera* is found in aphid saliva and has
76 been shown to trigger plant defenses and reduce aphid fecundity using transgenic plants
77 expressing GroEL (36,37). Virus-induced changes in plant defense or nutrients may alter aphid-
78 *Buchnera*-plant interactions, highlighting the need for additional research on virus-vector-
79 *Buchnera* interactions (38).

80 *Potato leafroll virus* (PLRV) is a positive sense ssRNA virus and the type member of the
81 genus *Polerovirus* (family *Luteroviridae*). PLRV is phloem limited and transmitted in a
82 circulative nonpropagative form. This means the virus particles will travel across the gut
83 membrane on specific receptors into the insect hemolymph. From here it will traverse to the
84 salivary gland and duct so that it may be injected back into the phloem tissue (9,39). Previous
85 studies have shown that aphid vectors prefer to settle on plants infected with PLRV and that
86 insect vectors have higher fecundity when feeding on these plants compared to controls (5,40–
87 42). Additionally, a recent study investigating aphid small RNAs (sRNAs), found that *M.*
88 *persicae* who fed upon PLRV infected plants and purified PLRV diets had significantly altered

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89 sRNA profiles, manipulated immune response, and differentially expressed *Buchnera* sRNAs
90 that are associated with tRNAs (43). The role of *Buchnera* tRNA associated sRNAs is largely
91 unclear, as their expression is conserved in divergent *Buchnera* taxa (44), they are differentially
92 expressed during aphid development (45) and when aphids feed on different host plant diets (46).
93 In consequence, the mechanisms mediating virus-aphid-*Buchnera* interactions are largely
94 unknown. Recently we demonstrated PLRV induces changes in plant nutrients and defenses in
95 infected host plant, (47) however, the impacts of these changes on symbiont-aphid interactions
96 are unknown. To address this lack of knowledge we examined changes in the transcriptome of
97 *M. persicae* with and without PLRV, *Buchnera* titer, and changes in aphid and *Buchnera*
98 transcripts from aphids feeding on PLRV-infected plants. By providing evidence that
99 nonpropagative circulative plant viruses can affect insect vectors through changes in the
100 transcriptome and alter *Buchnera* titer, our study will contribute to growing knowledge of the
101 insect microbiome at a plant-insect interface.
102
103 **Methods:**
104 **Plant and insect growth conditions**
105 *Solanum tuberosum* were propagated using leaf-bud cutting from cv. Désirée (48) in laboratory
106 experiments. Plants were grown in growth chambers under controlled conditions (25/23°C
107 day/night with a photoperiod of 16/8 h day/night). Non-viruliferous and viruliferous aphid
108 clones of a potato-adapted red strain of *Myzus persicae* were reared under controlled conditions
109 (25/23°C day/night with a photoperiod of 16/8 h day/night) on healthy potato. All experiments
110 were conducted in the same environmental chambers and conditions, so there were no

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111 environmental differences in treatments (25/23°C day/night with a photoperiod of 16/8 h
112 day/night).

113 **Pathogen infection**

114 *Agrobacterium tumefaciens* (LBA4404) containing the infectious clone of PLRV (49) was grown
115 at 28°C in in LB broth (+10 mM MgSO4), with kanamycin (50 µg/mL), carbenicillin (100
116 µg/mL) and rifampicin (50 µg/mL) for selection. After 24 hours, bacteria were centrifuged to
117 concentrate and resuspended in 10mM MgCl2. One week old *S. tuberosum* were inoculated at an
118 optical density (OD) of 0.70. Three weeks post infection, tissue was collected from all plants,
119 RNA was extracted using the SV Total Isolation Kit as per manufacturer's instructions
120 (Promega, Madison, WI, USA), and cDNA was synthesized using 1500 ng of total RNA and
121 random hexamers (20ng/µL) with the SMART® MMLV as per manufacturer's instructions
122 (Takara Bio USA, Mountain view, CA, USA). cDNAs were used in PCRs with PLRV specific
123 primers (F-5'ATGAGTACGGTCGTGGTT-3' and R-5'CTATTTGGGGTTTTGCAAAGC-3').
124 A set of uninfected potato cuttings were grown at the same time as the plants above to serve as
125 controls. After systemic plant infection was verified plants were immediately used in
126 experiments.

127

128 **RNAseq, qRT-PCR, and qPCR aphid experiments**

129 Five adult aphids were placed on the first fully expanded leaflet of three infected and on three
130 healthy plant. After 24 hours, all adults were removed and 20 larvae were left to develop. Seven
131 days later all aphids were at the same developmental stage and 10 young aphids were collected
132 into a tube from each plant (N=3 plants with 10 aphids per plant) and immediately frozen in
133 liquid nitrogen until use in RNAseq experiments. The entire experiment was repeated a second

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134 time for confirmation of RNAseq results using qRT-PCR and to examine the titer of the bacterial
135 symbiont, *Buchnera*, in aphids. For this experiment 5 aphids were collected for RNA extraction
136 and 5 aphids were collected for DNA extractions from each plant. We also prepared 6-7 plants
137 for each treatment instead of 3 (N = 6-7 with 5 aphids per plant), however all other methods were
138 the same.
139

140 **RNA and DNA isolation from aphids**

141 RNA was extracted from aphid tissue collected in the first two experiments as described above.
142 The RNA concentration and purity were measured using a NanoDrop. The integrity of RNA was
143 confirmed using the Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA). DNA was
144 extracted from aphid tissue collected in the second experiment using Cetyl trimethylammonium
145 bromide. The integrity of DNA was confirmed using an agarose gel. The DNA concentration and
146 purity were measured using a NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA).
147

148 **Library Preparation, and Sequencing.**

149 Sequencing libraries were prepared using a multiplexing library protocol (50). Briefly, oligo(dT)
150 25 Dynabeads were used to purify mRNA, which was then fragmented, and the first-strand
151 cDNA was synthesized using random primers, dNTP, and reverse transcriptase. The second-
152 strand was synthesized using a dUTP mix, DNA Polymerase I, and RNase H, ends repaired, and
153 adenylated. The cDNA fragments were ligated to adapters, selectively enriched by PCR, and
154 purified using the AMPure XP beads. The library quality was assessed using the Agilent
155 Bioanalyzer 2100 system and sequenced using an Illumina HiSeq 2000 instrument.

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156

157 **Read mapping, differential expressed gene (DEG) analysis, and Gene Ontology (GO)**
158 **classification**

159 RNA-Seq data were analyzed using RStudio (Version 1.1.383) and Bioconductor according to
160 Anders et al. (2013) with some modifications (48) (See Supplementary Figure S1). Sequence
161 quality was determined, trimmed, and poor-quality reads removed using ShortRead (52) and
162 FastQC (53). Reads were mapped to the *Myzus persicae* clone G006 genome v2.0 from
163 AphidBase (54) using TopHat2 (55). Mapped reads were assigned to genes and counted with
164 HTSeq (56) and normalized by size factors obtained from the negative binomial-based DESeq2
165 package (57). Gene annotation files for both were downloaded from NCBI. After normalization,
166 clusterization profiles of the samples were assessed by hierarchical clustering (with Euclidean
167 distance metric and Ward´s clustering method) and principal component analysis (PCA).
168 Differentially expressed genes (DEGs) between infected and control treatments were identified
169 using DESeq2 (57). Genes with False Discovery Rate (FDR)-corrected p-values ≤ 0.1 were
170 classified as differentially expressed. For Gene ontology (GO) analysis, Blast2GO software (58)
171 was utilized for annotation as previously described (59).

172

173 **qRT-PCR**

174 cDNA was synthesized using random hexamer (20ng/μL) and quantitative RT-PCR (qRT-PCR)
175 was performed. Transcript abundance was quantified for the *M. persicae* genes, *HSP68-like*
176 (MYZPE13164_G006_v1.0_000070430.1) and *M. persicae* cuticle protein5-like (*MPCP5-like)*
177 (MYZPE13164_G006_v1.0_000133030.2), and for the *Buchnera* genes, *argE*
178 *(BUMPUSDA_CDS00542), dnaK (BUMPUSDA_CDS00441),* and *groEL*

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179 *(BUMPUSDA_CDS00567),* using gene specific primers (Supplementary Table S1). qRT-PCR
180 reactions were carried out using SYBR green PCR master mix (Applied Biosystems, Carlsbad,
181 CA, USA), in an CFX384 instrument (Bio-Rad, Hercules, CA, USA). Three technical replicates
182 were performed for each individual sample, and a digital pipette was used for all pipetting.
183 Relative transcript abundance was calculated utilizing a standard curve produced from 10-fold
184 series dilution of cDNA synthesized from 1000 ng/µL of total RNA according to the standard
185 curve method (Applied Biosystems, Carlsbad, CA, USA). Technical replicates of raw CT values
186 were averaged and transcripts of interest were normalized to the house-keeping transcript
187 ribosomal protein L7 *rpl7* for aphids or the 50S ribosomal subunit gene *rpIN* for *Buchnera*, as
188 previously described (60–62).

189

190 ***Buchnera* titer**

191 *Buchnera* titer here is defined as the ratio of *Buchnera* single copy genes to aphid single copy
192 genes. To determine *Buchnera* titer in whole aphid bodies we used quantitative PCR (qPCR) and
193 measured the ratio of a single copy *Buchnera* gene (*rplN*) to a single copy aphid gene (*RPL7*).
194 qPCR reactions were carried out using SYBR green PCR master mix (Applied Biosystems,
195 Carlsbad, CA, USA), in the CFX384 instrument (Bio-Rad, Hercules, CA, USA). Reactions were
196 performed in triplicate for each sample, and the average was used for analysis. Relative
197 abundance was calculated utilizing a standard curve produced from 10-fold serial dilution of
198 DNA.

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199

200 **Statistical analyses**

201 RNAseq data analyses were performed as described above. All statistical analyses for qRT-PCR
202 and qPCR were determined using a $P < 0.05$. Analysis of variance (ANOVA) was used to
203 determine significant difference in transcript abundance. For *Buchnera* titer single factor
204 ANOVA was used to determine difference in relative abundance. The statistical analyses were
205 performed using JMP 8 software (SAS Institute, Cary, NC, USA).

206

207 **Results:**

208 **Differential gene expression in the presence of PLRV.**

209 Over 150 million paired-end reads were obtained through Illumina sequencing, with an average
210 of 125,815, 247 100-bp reads per library (Table 1, Fig 1A). About 73% of the reads mapped to
211 the *M. persicae* reference genome, with around only 27% being uniquely mapped (Table 1, Fig
212 1B). In order to examine biological variability, a principal component analysis (PCA) of the
213 normalized count data was performed (Fig 1C). The first component of variance separated
214 samples by treatments and accounted for 54% of the variance. Hierarchical clustering confirmed
215 PCA results in visual representation of DEG expression (Fig 1D). The transcriptome of
216 viruliferous *M. persicae* was compared to the transcriptome of virus-free *M. persicae* using the
217 negative binomial-based DESeq2 (57). Overall, 96 differentially expressed genes (DEGs) were
218 detected using an FDR adjusted p-value ≤ 0.05 and log2 fold change (FC) ≥ 0.5, however, by
219 relaxing our FDR adjusted p-value to ≤ 0.1 we were able to include 38 additional DEGs (134
220 DEGs total included; Fig 1E; Supplementary Table S2). The presence of PLRV in the aphid
221 vector caused a down-regulation of 70 genes and an up-regulation of 64 genes (Fig 1F; FDR

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222 adjusted p-value ≤ 0.1 and log2 fold change (FC) ≥ 0.5). Overall, 0.8 % of the aphid genome was
223 significantly impacted by the presence of PLRV.

224 **Functional roles of differentially expressed genes**

225 Gene ontology (GO) enrichment analyses were performed with the DEGs from each treatment to
226 identify functions and pathways disturbed in aphids carrying PLRV. One or more gene ontology
227 terms were assigned to each transcript from biological processes, molecular functions, and
228 cellular compartments term using Blast2GO functional gene annotation (Conesa et al. 2005). The
229 134 DEGs were assigned to functional GO terms within the three categories, including 125
230 biological processes, 118 molecular functions, and 148 cellular compartments. Of the 134 DEGs,
231 53 (39.55% of total DEGs) were classified as "uncharacterized proteins." The majority of DEGs
232 assigned to biological processes were categorized as metabolic processes (41%), cellular-protein
233 processes (11%), and oxidation-reduction processes (11%) (Fig 2A). As for DEGs assigned to
234 molecular functions, almost half were associated with catalytic activity (33%) or nucleic acid
235 binding (21%) (Fig 2B). Within the cellular component category, 24% were related to the
236 membrane and 24% were related to intracellular locations (Fig 2C).

237 Next each DEGs was annotated using a single Blast2GO consensus description. Many of
238 the genes up-regulated in PLRV aphids were related to cuticle formation and development (16%)
239 and catalytic activity (16%), however the majority of up-regulated transcripts were
240 uncharacterized (31%; Fig 3A). The largest groups of down-regulated genes in PLRV aphids
241 were related to histones (10%), catalytic activity (10%), transmembrane transport (9%),
242 proteolysis or protein ubiquitination (7%), and nucleic acid binding and metabolic processes
243 (7%; Fig 3B). A significant proportion of the down-regulated transcripts in PLRV aphids were
244 also uncategorized (47%). The most highly expressed DEGs included transcripts related to

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245 cuticle formation and development and 4C1-like cytochrome P450s (Table 2). The most down-
246 regulated transcripts in PLRV aphid were related to histones and histone modifying proteins
247 (Table 3).

248 **Validation of aphid transcripts via RT-qPCR**

249 To validate the RNAseq analysis a separate experiment was performed using the same
250 experimental design and the transcript abundance of one up-regulated gene
251 (MYZPE13164_G006_v1.0_ 000133030.2 (*MPCP5-like*)) and one down-regulated gene
252 (MYZPE13164_G006_v1.0_ 000070430.1 (*HSP68-like*)) was measured using qRT-PCR (bolded
253 genes in Table 2 and 3). Consistent with the RNA-seq data, abundance of the *MPCP5-like*
254 transcript was significantly higher in viruliferous *M. persicae* compared to virus-free controls
255 (10.045, 2.017, relative expression respectively; $p = 0.019$; Fig. 4A). Abundance of the *HSP68-*
256 *like* transcript was significantly lower in viruliferous *M. persicae* when compared to virus-free
257 controls (1.44, 5.51, relative expression respectively; $p < 0.01$) (Fig 4A-B).

258

259 **The impact of PLRV on *Buchnera aphidicola* titer**

260 *Buchnera* has been previously implicated in transmission of PLRV and other luteoviruses
261 (26,28,30,34,63), however *Buchnera* titer and coding sequence transcripts have not been
262 examined in aphids carrying PLRV. From our experiments, *Buchnera* titer was ~1.5 times higher
263 for virus-free aphids compared to aphids carrying PLRV (ratios 6.42, 4.20 respectively; p =
264 0.037; Fig 5A). To investigate the potential mechanisms mediating decreases in *Buchnera* titer
265 we measured abundance of two transcripts related to stress, *dnaK* (64,65) and *groEL* (65,66), and
266 one transcript related to metabolism, *argE* (45). Abundance of all three transcripts were reduced
267 in aphids carrying PLRV compared to controls. Viruliferous aphids had 36.68% less *argE*

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268 transcripts (p = 0.026), 16.67% less *groEL* transcripts (p = 0.024), and 18.77% less *dnaK*
269 transcripts (p = 0.046) compared to that of the virus free aphids (Fig 5B-D).
270

271 **Discussion:**

272 The main focus of this paper was to examine the effect that *Potato leafroll virus* has on the
273 transcriptome of *M. persicae,* and their primary endosymbiont *Buchnera aphidicola*. The largest
274 category of known up-regulated transcripts in viruliferous aphids compared to controls were
275 related to the cuticle and cuticle development. Insect cuticles are largely composed of a protein
276 matrix embedded with chitin filaments (67). Cuticle proteins (CPs) have been shown to be
277 involved in general development, molting, transmission of non-persistent viruses, and insecticide
278 resistance through changes cuticle permeability (33,68–71). In *Acyrthosiphon pisum*, 19 CPs
279 were found to be regulated by photoperiodism and suspected to be involved in the transition
280 from asexual to sexual production (72). Further, cuticle proteins have been implicated as
281 potentially facilitating transmission of *Barley yellow dwarf virus* (BYDV-GPV), *Cereal yellow*
282 *dwarf virus* (CYDV-RPV), and *Turnip yellows virus* (TuYV), three related Luteoviridae viruses
283 (73–75) . Whilst we cannot know the function of changes in CP transcripts in PLRV-aphid
284 interactions from these experiments, these genes represent promising targets for further
285 investigation.

286 In addition to many cuticle related proteins, five *cytochrome P450s* genes were
287 significantly up-regulated in viruliferous aphids compared to controls. Cytochrome P450s play
288 important roles in hormones and pheromones metabolism but are more famous for their roles in
289 the metabolism of insecticides and host plant chemicals. Polyphagous insects, like *M. persicae,*
290 encounter many different hosts and tend to have high numbers P450-based metabolism of

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291 allelochemicals compared to more specialized aphids (76). Previous work has shown that a
292 *cytochrome 450 gene* (*CYP6CY3*) was found to increase nicotine tolerance and aphid host
293 adaptation (77,78). It has been previously hypothesized that upregulation of p450s could help
294 insect vectors tolerate less desirable hosts which could be beneficial to the virus (79).

295 Transcripts encoding a heat shock protein (HSP68-like) was among the most down-
296 regulated in virluiferous aphids compared to controls. HSP68 is a member of the HSP70 family,
297 which are important chaperone proteins that are known to be up-regulated in response to stress.
298 One study found that the *HSP70* from *Bemisia tabaci* is up-regulated after acquisition of *Tomato*
299 *yellow leaf curl virus* (TYLCV) (80). They went on to show that HSP70 protein can directly
300 interact with TYLCV using *in vitro* studies and that they co-localize together in insect midgut
301 cells using *in situ* hybridization. The authors suggest HSP70 may play an inhibitory role in virus
302 transmission, as transmission was reduced when whiteflies were fed HSP70 antibodies. Because
303 *HSP68-like* transcripts were down-regulated in aphids carrying PLRV in our study, it's tempting
304 to speculate that this may increase PLRV transmission. Porras et al. demonstrated that BYDV-
305 PAV, a strain that is only transmitted by *Rhopalosiphum padi* (bird-cherry oat aphid), up-
306 regulated the abundance of three *HSP70* transcripts in the aphid vector. The authors found
307 BYDV infection increases plant surface temperature and aphid heat tolerance, suggesting a
308 protective role of HSP70 proteins in virus-aphid-plant interactions (81). Although it is not known
309 if PLRV increases plant surface temperature and vector heat tolerance, it has been shown that
310 potato plants kept at higher temperatures are more susceptible to PLRV than compared to lower
311 temperatures (82). Also aphid acquisition and transmission at higher temperatures has previous
312 resulted in higher transmission rates compared to lower temperatures (83), however at very high

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313 temperatures differences were reduced (84). It is not known how decreases in *HSP68-like*
314 transcripts in aphids carrying PLRV in our study may alter aphid heat tolerance.
315 Several histone genes were down-regulated in viruliferous *M. persicae* compared to
316 controls in this study. Histones are involved in DNA organization and regulation of gene
317 expression (85), but have also been shown to be induced in response to stress and starvation (86).
318 Histone depletion can lead to changes to an open chromatin configuration and large scale shifts
319 in expression, however it should be noted histone depletion is also associated with DNA damage
320 (87). In a recent study investigating small RNAs (sRNAs), *M. persicae* who fed upon PLRV
321 infected plants and purified PLRV diets had significantly altered sRNA profiles and immune
322 responses. Ultimately, the significance of this response is unknown, and further investigation is
323 necessary to understand potential impacts of the downregulation of these genes.
324 In this study there was a significant reduction of *Buchnera* titer and *Buchnera* gene
325 expression of three genes (*dnaK*, *groEL,* and *argE*) in aphids carrying PLRV compared to
326 control aphids. In general, gene regulation at the mRNA level in *Buchnera* is thought to be
327 minimal because *Buchnera* transcription factors are reduced (88) and very few transcriptional
328 responses had been observed previously (17). Only two transcription initiation factors (σ32 and
329 σ70), the heat shock and housekeeping transcription factors, respectively, remain in *Buchnera*
330 Myzus's genomes (89) similar to other *Buchnera* taxa (90). The housekeeping sigma factor
331 (σ70) initiates transcription of *argE* which is regulated by the repressor *argR* when bound to
332 arginine in *Escherichia coli* (91). Similar to other Buchnera taxa *Buchnera* Myzus's genomes
333 (89) has lost the repressor argR so it is unclear how this gene is down-regulated in virus infected
334 aphids compared to un-infected aphids. The other two *Buchnera* genes (*dnaK* and *groEL*) that
335 were down-regulated in this study in aphids carrying PLRV compared to control aphids are

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336 associated with the heat shock regulon (90). Moreover, these *Buchnera* genes still retain
337 recognizable $\sigma 32$ promoter sites up-stream of *dnaK* and *groEL* in the Myzus *Buchnera* G006
338 genome (NCBI Reference Sequence: NZ_MJNC01000001; Supplemental Table 3) similar to
339 other *Buchnera* taxa (90). The $\sigma 32$ heat shock response is highly conserved in bacteria and is
340 initiated in response to stress, such as heat shock or other environmental stressors that destabilize
341 proteins (92). In general, compared to free-living bacteria, *Buchnera* only modestly up-regulates
342 genes that still retain the upstream $\sigma 32$ promoter sites during heat shock (90). In this study it is
343 unclear how PLRV is either directly or indirectly dampening *Buchnera*'s expression of *dnaK* and
344 *groEL* and if it is through a similar mechanism that is also down-regulating the aphid's stress
345 response genes including *Hsp70*.

346 A decrease in *Buchnera* titer has previously been associated with different aphid clones
347 (93), plant diets (16), increasing aphid nymphal age (94,95), and heat shock (95–97). For
348 example, a mutation in the promoter region of the heat shock gene (*ibpA*) results in the reduction
349 of *Buchnera* titer (96). Given that the experiments in this study were conducted in the same
350 environment at the same temperature it is highly unlikely that the control aphids experienced
351 heat shock compared to virus infected aphids. Instead, we hypothesize that the virus PLRV is
352 reducing *Buchnera*'s ability to up-regulate genes that are associated with the heat shock regulon
353 and this may lead to increased stress and lysing of *Buchnera* cells and ultimately a reduction of
354 *Buchnera* titer. For example, most obligate pathogens and symbionts, including *Buchnera,*
355 overexpress the protein GroEL during non-heat shock conditions to rescue misfolded proteins
356 (98). Alternatively, as PLRV-infected plants have higher concentrations of free amino acids (47)
357 and we cannot discount the indirect impacts of host plant changes on *Buchnera* titer in our
358 experimental design, similar to Zhang et al. (16) where a change in host plant diet influenced

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359 *Buchnera* titer. Buchnera sRNA's that are hypothesized to regulate *Buchnera* gene expression at
360 the post-transcriptional level have been observed to be differentially expressed when aphids feed
361 on host plants that vary in essential amino acids (46). In turn, aphids carrying PLRV may obtain
362 higher levels of essential amino acids from virus infected plants and as such *Buchnera* genes that
363 are involved in arginine biosynthesis, such as *argE*, are down-regulated compared to control
364 aphids feeding on un-infected plants with lower amounts of essential amino acids.

365 In other insect-plant pathogen systems plant pathogens are known to modulate obligate
366 symbiont titer. For example, in whiteflies Portiera titer is modulated by the co-occurrence of its
367 facultative symbiont *Rickettsia* and the Tomato Yellow Leaf Curl Virus (99). In a second system,
368 the obligate symbionts *Carsonella* and *Profftella* of the psyllid *Diaphorina citri* decrease in titer
369 when the plant pathogen *Ca*. Liberibacter asiaticus infects *D. citri* male adults, whereas no
370 significant difference was found in infected female adults (100,101). In adult females' ovaries,
371 *Carsonella* and *Profftella* titer increased when infected by *Ca.* L. asiaticus. The latter authors
372 hypothesized that endosymbionts titer increases as a part of the psyllid's immune response
373 and/or response to altered plant nutrition by *Ca.* L. asiaticus infection (100).

374 In general, this work improves our understanding of the relationships that exist between
375 hosts, viruses, vectors, and endosymbionts, but it also opens up more questions regarding the
376 complexity and depth of these relationships. Aphids and bacterial endosymbionts may benefit
377 from relationships with plant-infecting viruses indirectly or directly but additional studies are
378 needed. Although it is known that *Buchnera* titer and gene expression responses vary with aphid
379 linages (102), it is not known how this is impacted by long term associations with plant-infecting
380 viruses. In regions where virus pressure is high or where poor hosts dominate, aphids may more
381 often be associated with plant infecting viruses. Given the mounting evidence of virus

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382 manipulation of insect vectors, this could have lasting impacts on the population structures of
383 these insect vectors and their obligate endosymbiont.
384

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389

390 **Author contributions**

391 CLC conceived the project. MFP and CLC designed the research. MFP and CLC performed
392 research and analyzed the data. AKH, MFP, and CLC interpreted the data. AKH, MFP, and CLC
393 wrote the article.
394

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676 **Figure Legends**

677 **Fig 1. Overview of *Myzus persicae* transcriptome after *Potato leafroll virus* (PLRV)
678 acquisition.** (A) Number of paired-end reads generated for each library by Illumina HiSeq
679 sequencing. The dashed line represents the average of paired-end reads from all 6
680 libraries. (B) Proportion of uniquely mapped, multimapped, and unmapped reads obtained for
681 each library. Reads were mapped in the *Myzus persicae* clone G006 genome (AphidBase).
682 (C) Principal component analysis of normalized count data from all samples. (D) Hierarchical
683 clustering analysis of normalized count data z-scores exhibited by differentially expressed genes
684 (DEGs) within each sample. (E) Volcano-plots of –log10p and log2FC exhibited by each gene in
685 viruliferous aphids compared to controls. Up- and down-regulated genes are presented in red and
686 green, respectively. (F) Numbers of up- and down-regulated DEGs in viruliferous aphids in
687 comparison to control aphids. DEGs were identified using DESeq2 and defined by $|\log2FC| \geq 0.5$
688 and false discovery rate (FDR)-corrected p-value $\leq$ 0.1. Control (aphids without virus);
689 Viruliferous (aphids carrying PLVR). FC, fold-change; p, FDR-corrected p-value.

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691 **Fig 2. Blast2GO Gene Ontology of DEGs arranged by functional categories.** (A) Biological
692 processes (BP), (B) Molecular Function (MF), and (C) Cellular Component (CC). The predicted
693 gene functions of differentially expressed genes as assigned by Blast2GO at level 2-3 in each
694 aforementioned category. Each DEG may be assigned to one or more GOterm, with a total of
695 391 GOterms from the three functional groups assigned to the 134 DEGs.

696 **Fig 3. Blast2GO annotation for up-regulated DEGs and down-regulated DEGs in aphids**
697 **carrying PLRV compared to controls.** The consensus description predicted by Blast2GO of
698 the (A) 64 up-regulated DEGs and the (B) 70 down-regulated DEGs.

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700 **Fig 4: Relative transcript abundance of two genes in *Myzus persicae* with and without**
701 ***Potato leafroll virus* (PLRV).** (A) A cuticle related protein (MPCP5-like) was significantly up-
702 regulated in expression in individuals with PLRV compared to virus free controls. (B) A
703 predicted heat shock protein (HSP68-like) was significantly down-regulated in expression in
704 individuals with PLRV compared to controls. Transcripts were measured relative to a
705 housekeeping gene *RPL7*. Significant differences were calculated using an ANOVA (*$P < 0.05$;
706 Error bars represent ±SEM).

707 **Fig 5. *Buchnera aphidicola* titer and transcript changes in *Myzus persicae* with *Potato***
708 ***leafroll virus* (PLRV).** (A) Ratio of a single copy *Buchnera* gene *rplN* relative to a single copy
709 aphid gene *RPL7,* demonstrates *M. persicae* with PLRV have a decreased *Buchnera aphidicola*
710 titer relative to control aphids. (B-D) *Buchnera* transcripts *groEL*, *dnaK*, and *argE* relative to the
711 *Buchnera* gene *rplN* housekeeping gene. All three transcripts were down-regulated in

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712 viruliferous *M. persicae* compared to virus free controls. Significant differences were calculated
713 using an ANOVA (*$P < 0.05$; Error bars represent ±SEM).
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717 **Tables**
718 **Table 1: RNAseq Stats.**

Sample Name	Total paired-end reads	Total alignments	Aligned	Unique paired	Non-unique paired
Control 33	40,676,571	30,990,077	76.17%	28.52%	47.67%
Control 34	18,942,570	13,440,120	70.80%	25.89%	45.07%
Control 35	22,651,445	16,478,122	72.49%	25.93%	46.82%
PLRV 36	25,343,473	18,306,992	72.12%	26.30%	45.94%
PLRV 37	23,743,718	17,496,940	73.63%	27.15%	46.54%
PLRV 38	23,533,704	17,410,337	73.78%	27.05%	46.93%

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734 **Table 2: Most highly up-regulated *M. persicae* genes that were characterized* in aphids**
735 **carrying PLRV compared to controls.** DEGs determined by adjusted p value < 0.1 and
736 described by Blast2GO. Gene ID corresponds to MYZPE13164_G006_v1.0_XXXXXXXXX.X
737 found on AphidBase.org. Regulation of bolded transcripts were validated in a separate
738 experiment.
739 *20 uncharacterized genes were up-regulated (See Supplementary Table S2)

Putative function	Gene ID	Blast2GO consensus description	p-val	log2
Cytochrome P450	000087490.2	cytochrome P450 4C1-like	1.30E-05	2.34
	000113270.1	cytochrome P450 4C1-like	1.90E-05	2.21
	000087490.3	cytochrome P450 4C1-like	3.10E-09	2.16
	000111320.1	cytochrome P450 6k1-like	1.10E-04	0.95
Cuticle related	**000133030.2**	**Adhesion plaque protein, chitin binding**	**4.60E-05**	**2.24**
	000133030.1	Adhesion plaque protein, chitin binding	1.90E-04	1.91
	000086070.1	endocuticle glycoprotein in abdomen	3.00E-07	1.05
	000079280.1	osiris 20-like	1.80E-06	0.95
	000103820.1	Adhesion plaque protein, chitin binding	6.60E-06	0.88
	000103820.2	Adhesion plaque protein, chitin binding	1.50E-06	0.87
	000079260.1	osiris 18	9.40E-05	0.85
	000084640.1	glycine and glutamine-rich	1.40E-05	0.77
	000047580.1	Myzus persicae tentative cuticle protein	2.60E-04	0.74
Kinase Inhibitor repressors	000156640.1	52 kDa repressor of kinase inhibitor-like	7.10E-05	0.88
	000156640.2	52 kDa repressor of kinase inhibitor-like	3.70E-04	0.79
	000156640.4	52 kDa repressor of kinase inhibitor-like	3.50E-04	0.78
Kinases	000073070.1	alpha- kinase 1-like	2.20E-06	0.78
	000137500.2	serine threonine- kinase (NEK3)	2.20E-06	0.76
Hydrolase	000181580.1	N-acetylmuramoyl-L-alanine amidase-like	4.40E-04	0.93
Transcription factor	000125820.1	transcription factor A2 (mab3-liked)	1.10E-05	1.94
Zinc Transport	000174630.1	39S ribosomal mitochondrial	8.60E-05	0.77
Membrane	000137380.1	histidine-rich glycoprotein	3.00E-05	0.90
Cell organization	000090710.1	cytoskeleton-regulatory complex (pan1-like)	4.80E-04	1.268
	000021370.2	microtubular process (CFA58-like)	4.70E-04	1.265

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	000189110.1	actin reorganization (WAS-like)	4.50E-04	0.83

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742 **Table 3: Most highly down-regulated *M. persicae* transcripts that were characterized* in**
743 **aphids carrying PLRV compared to controls.** DEGs determined by adjusted p value < 0.1 and
744 described by Blast2GO. Gene ID corresponds to MYZPE13164_G006_v1.0_XXXXXXXXX.X
745 found on AphidBase.org. Regulation of bolded transcripts were validated in a separate
746 experiment.
747 *33 uncharacterized genes were down-regulated (See Supplemental Table S2)

Putative function	Gene	Blast2GO consensus description	p-value	log2
Histones	000100490.1	histone H3	5.06E-05	-2.58
	000100610.1	histone H3	2.69E-04	-2.14
	000100770.1	histone H3	1.04E-04	-1.74
	000100600.1	histone H2A	1.38E-04	-1.75
	000092680.1	histone H2A-like	9.76E-05	-1.47
	000100590.1	histone H2B-like	7.37E-05	-1.18
	000100620.1	histone H4	3.02E-04	-0.96
Histone Modifying	000163990.2	glycine-rich DOT1-like	7.33E-05	-0.81
	000163990.1	glycine-rich DOT1-like	1.97E-04	-0.76
ubiquitination	000119640.3	E3-ubiqutin ligase RNF19B-like	6.08E-06	-0.88
	000119640.1	E3-ubiqutin ligase RNF19B-like	1.42E-05	-0.81
	000119640.2	E3-ubiqutin ligase RNF19B-like	2.34E-05	-0.78
Hydrolase	000133360.1	serine carboxypeptidase	1.10E-04	-1.58
	000083200.2	Arylsulfatase B-like	4.69E-04	-1.11
	000083200.1	Arylsulfatase B-like	3.25E-04	-1.02
	000200070.1	Thioesterase (THEM6-like)	1.13E-04	-0.82
Response / Immunity	000071560.1	Protease inhibitor (Papain inhibitor)	2.91E-04	-2.46
	000070430.1	**Heat shock 68-like**	**4.85E-04**	**-1.88**
	000193260.2	G- coupled receptor Mth-like 3	2.03E-04	-0.80
Transport	000029670.1	dynein intermediate chain-like	1.90E-05	-1.01
	NRF6	Lipid transport (NRF6-like)	5.18E-05	-0.85
	000203490.1	zinc finger C3H1 type-like 2-A	1.03E-04	-0.86
	000072950.2	Sugar transport (TRET1-like)	6.74E-06	-0.81
nucleic acid metabolism	000036830.1	DNA integration (pol poly retrotransposon-related)	1.86E-04	-0.89
	000012610.1	mRNA catabolic process (BRISC/BRCA1-A complex-Like)	5.14E-04	-0.84

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752 **Supporting Information**

753 Supplementary Figure S1: Explanation of process

754 Supplementary Table S1: Primer Table

755 Supplementary Table S2: RNAseq significance list

756 Supplementary Table S3: Conserved σ32 -10 and -35 binding sites in *Escherichia coli* and

757 *Buchnera* taxa

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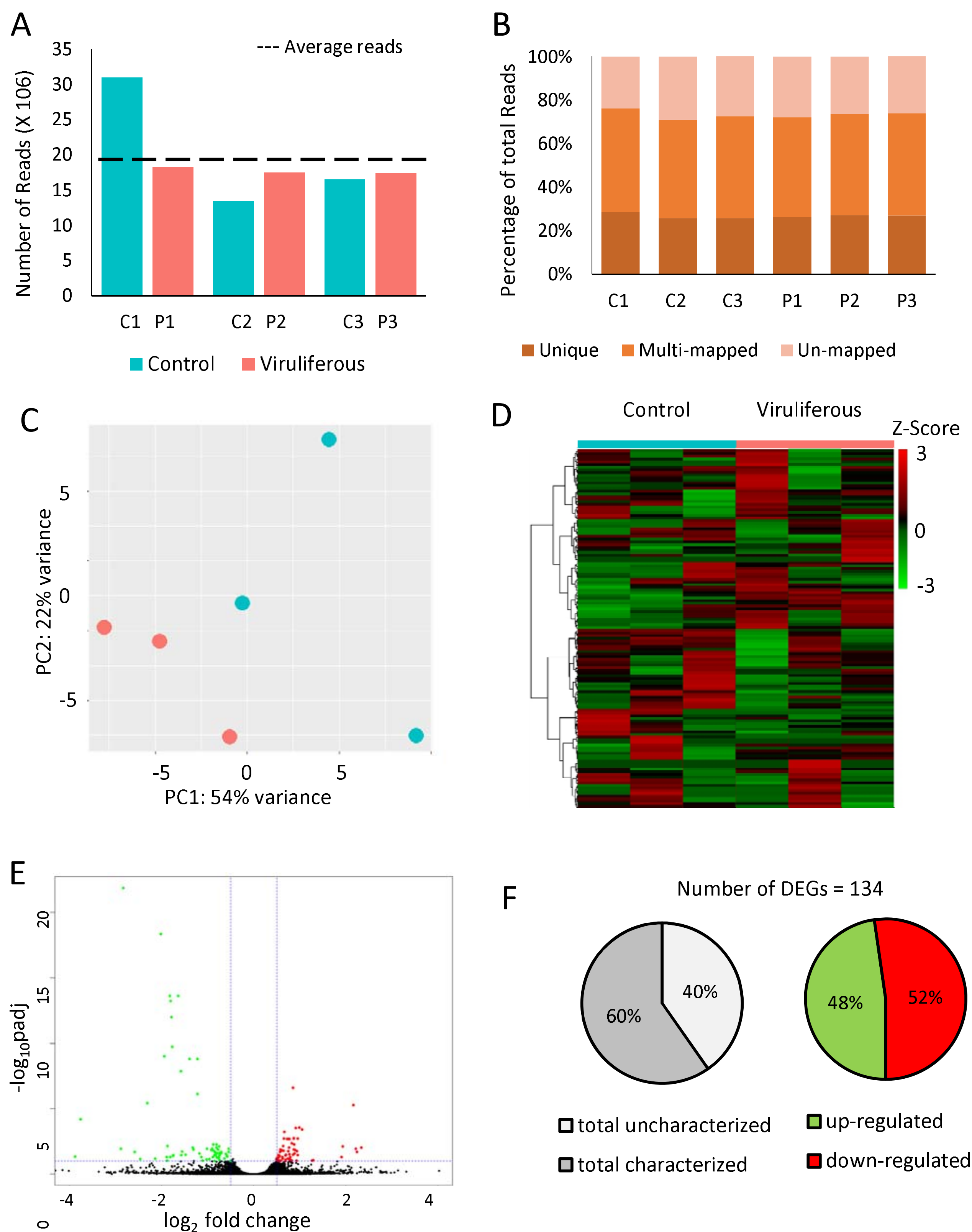

Figure 1: Overview of *Myzus persicae* transcriptome after *Potato leafroll virus* (PLRV) acquisition.

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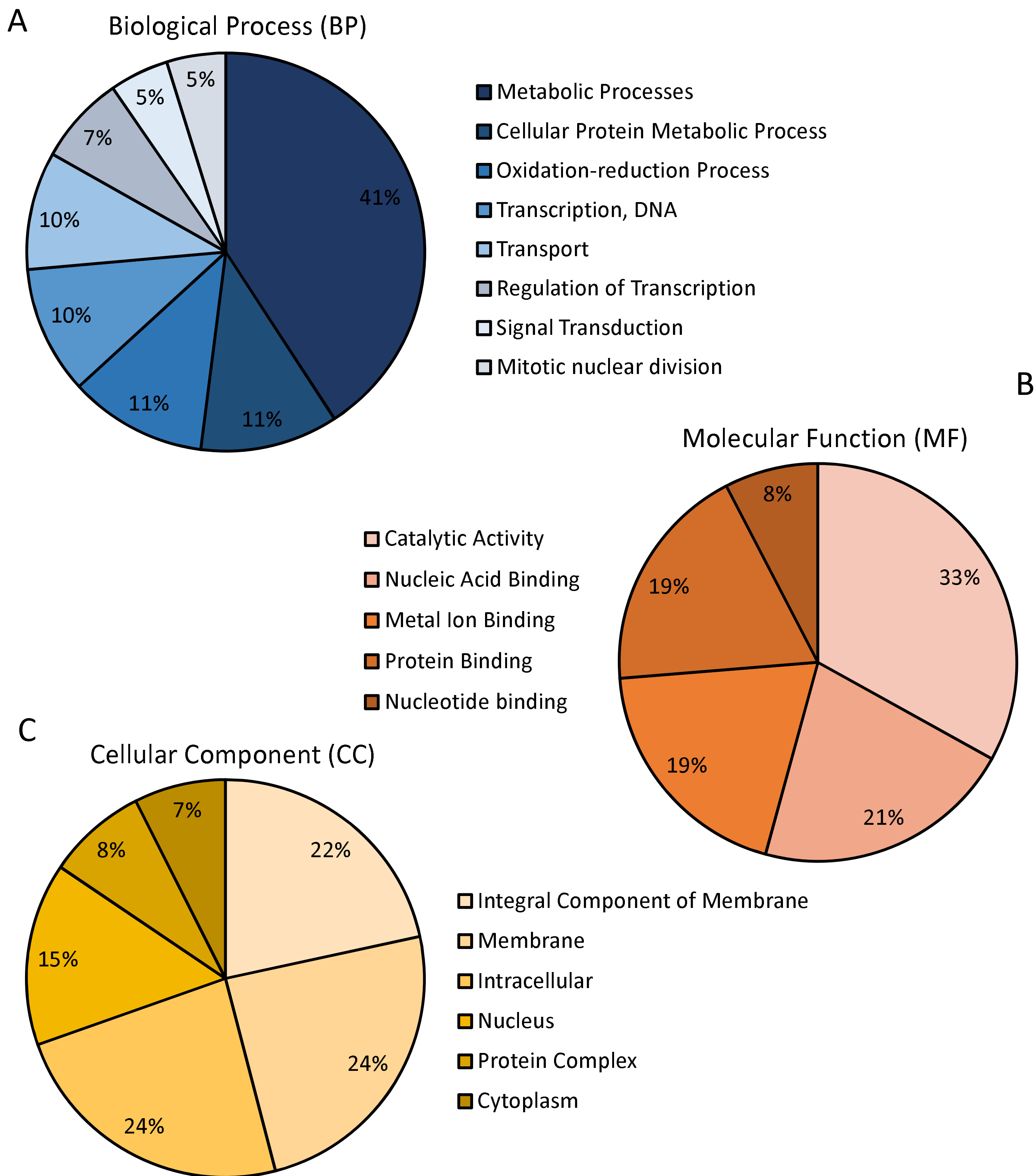

Figure 2: Blast2GO Gene Ontology of DEGs arranged by functional categories.

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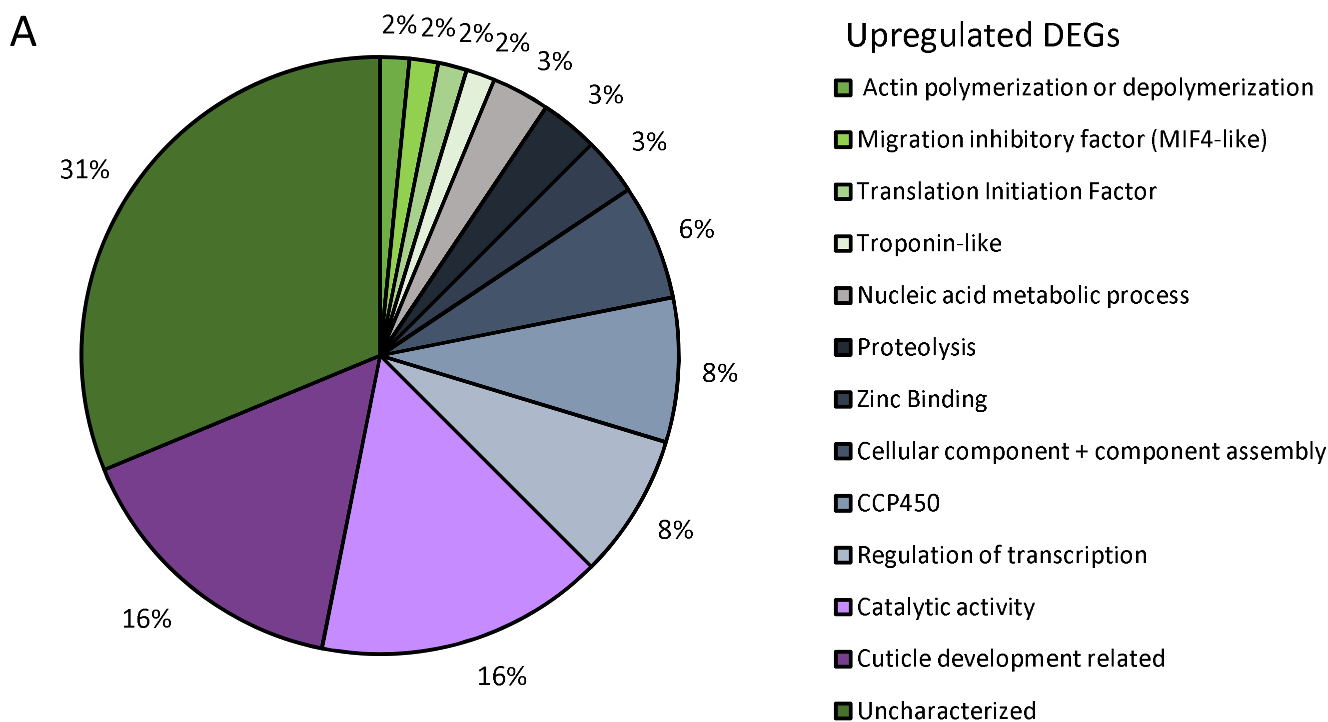

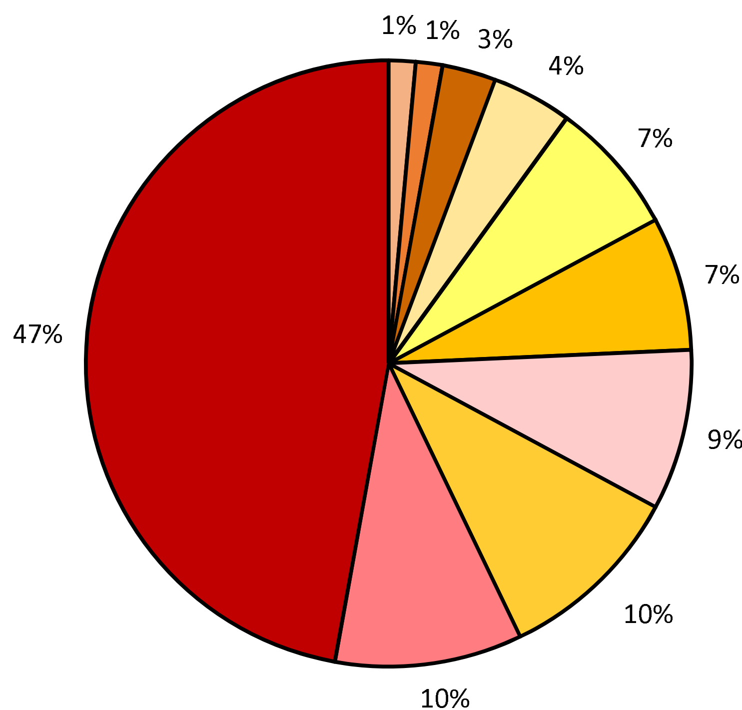

Figure 3: Blast2GO annotation for the up-regulated DEGs and down-regulated DEGs in aphid carrying PLRV compared to controls.

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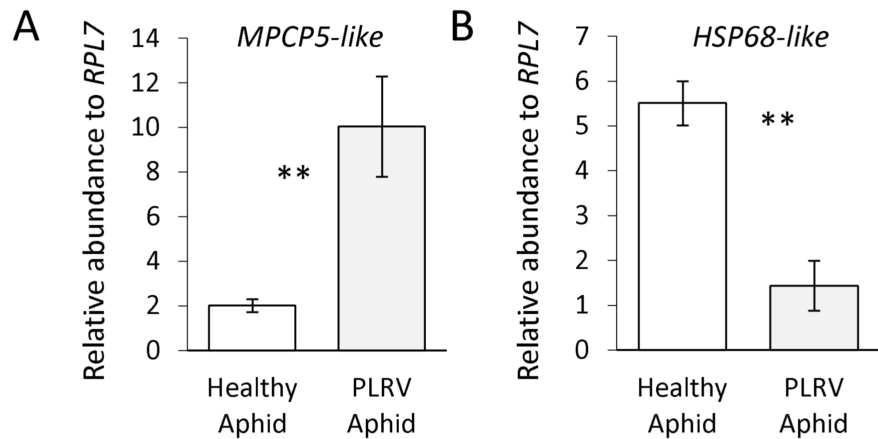

Fig 4: Relative transcript abundance of two genes in *Myzus persicae* with and without *Potato leafroll virus* (PLRV). (A) A cuticle related protein (MPCP5-like) was significantly up-regulated in expression in individuals with PLRV compared to virus free controls. (B) A predicted heat shock protein (HSP68-like) was significantly down-regulated in expression in individuals with PLRV compared to controls. Transcripts were measured relative to a housekeeping gene *RPL7*. Significant differences were calculated using an ANOVA (*$P < 0.05$; Error bars represent ±SEM).

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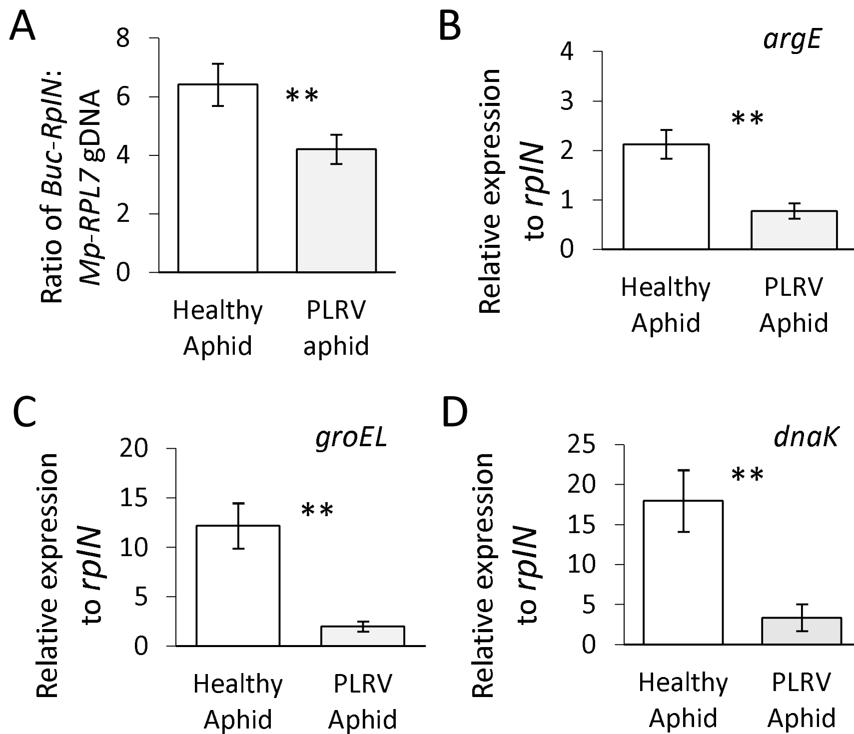

- **Fig 5. *Buchnera aphidicola* titer and transcript changes in *Myzus persicae* with *Potato leafroll virus* (PLRV).** (A) Ratio of a single copy *Buchnera* gene *rplN* relative to a single copy aphid gene *RPL7,* demonstrates *M. persicae* with PLRV have a decreased *Buchnera aphidicola* titer relative to control aphids. (B-D) *Buchnera* transcripts *groEL*, *dnaK*, and *argE* relative to the *Buchnera* gene *rplN* housekeeping gene. All three transcripts were down-regulated in viruliferous *M. persicae* compared to virus free controls. Significant differences were calculated using an ANOVA (**P* < 0.05; Error bars represent ±SEM).

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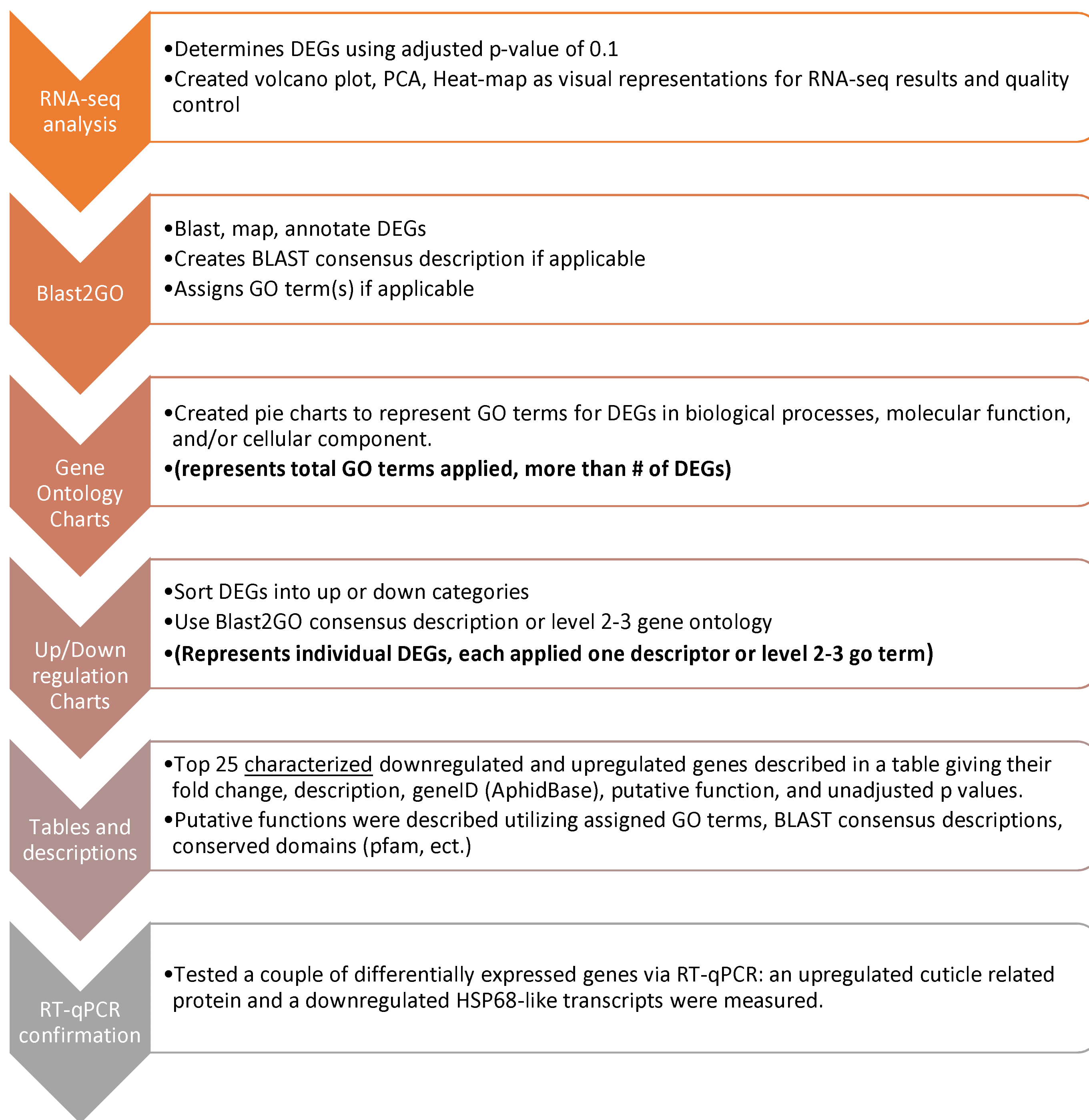

Figure S1: Explanation of process