

Supplementary Table S1. List of primers and probes used in this work. T7 promoter sequence is in bold. Fluorophore reporter and quencher are in italic.

Application	Primer name	5'–3' Sequences	Target gene
dsRNA synthesis	Eva_T7ATPsynBetaf ¹	TAATACGACTCACTATAGT TACGGCCAGATGAACGAGCC	ATP synthase β
	Eva_T7ATPsynBetar ¹	TAATACGACTCACTATAGGG ACACCACGAGCAATGTTG	
	T7GFPf ¹	TAATACGACTCACTATAGC TTTTCACTGGAGTTGTCCC	Green Fluorescent Protein (GFP)
	T7GFPPr ¹	TAATACGACTCACTATAGG TTTGTGTCCGAGAATGTTC	
qPCR (insect target gene)	ATPβFw622 ¹	CGCTTTACTCAGGCTGGTTC	ATP synthase β
	ATPβRv792 ¹	GTCATCAGCTGGCACGTAGA	
qPCR (insect housekeeping gene)	EF1-α_F215 ¹	CCATCGACATTGCCCTGTGG	elongation factor-1α
	EF1-α_R325 ¹	CCTGTGAGGTTCCAGTGATCATG	
	GST1_F257 ¹	CCAAGGACCCCAAGAAGCGA	glutathione S-transferase
	GST1_R369 ¹	TGGCGCTCCTCCAAACATCA	
qPCR (phytoplasma gene)	CYS2Fw ²	AGGTTGAACGGCCACATTG	Phytoplasma 16Sr RNA
	CYS2Rv ²	TTGCTCGGTCAGAGTTTCCTC	
	CYS2Probe ²	<i>FAM-ACACGGCCCAA</i> ACTCCTACGGGA- <i>TAMRA</i>	
qPCR (insect gene)	GapFw632 ³	ATCCGTCGTCGACCTTACTG	glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
	GapRv819 ³	GTAGCCCAGGATGCCCTTC	
	GapEvProbe ³	<i>HEX-ATATCAAGCCAAG</i> TCAAGGAGGC- <i>BHQ1</i>	

¹ Abbà, S.; Galetto, L.; Ripamonti, M.; Rossi, M.; Marzachi, C. RNA interference of muscle actin and ATP synthase beta increases mortality of the phytoplasma vector *Euscelidius variegatus*. *Pest. Manag. Sci.* **2019**, *75*, 1425–1434, doi:10.1002/ps.5263.

² Marzachi, C.; Bosco, D. Relative quantification of chrysanthemum yellows (16Sr I) phytoplasma in its plant and insect host using real-time polymerase chain reaction. *Molecular Biotechnology* **2005**, *30*, 117–128, doi:10.1385/MB:30:2:117.

³ Ottati, S.; Persico, A.; Rossi, M.; Bosco, D.; Vallino, M.; Abbà, S.; Molinatto, G.; Palmano, S.; Balestrini, R.; Galetto, L.; et al. Biological characterization of *Euscelidius variegatus* iflavivirus 1. *Journal of Invertebrate Pathology* **2020**, *173*, 107370, doi:10.1016/j.jip.2020.107370.

Supplementary Table S2. Mean normalized relative expression of ATP synthase β transcript \pm standard error of the mean (SEM) measured in head/body/whole insect of *Euscelidius variegatus* samples at 15, 22 and 37 days post injection (dpi) of dsRNAs targeting ATP synthase β or green fluorescent protein (GFP).

Days post injection	Target of dsRNAs	Sample type	Mean ATP synthase β expression \pm SEM (N)
15	ATP synthase β	Head	7.87E-03 \pm 1.86E-03 (10)
		Body	1.97E-02 \pm 9.03E-03 (10)
	GFP	Head	7.37E-01 \pm 1.06E-01 (10)
		Body	1.05 \pm 1.10E-01 (9)
22	ATP synthase β	Whole body	2.08E-02 \pm 2.21E-03 (92)
	GFP		2.81 \pm 4.53E-01 (79)
37	ATP synthase β	Whole body	2.25E-02 \pm 5.19E-03 (14)
	GFP		2.71 \pm 4.42E-01 (14)

Supplementary Table S3. Number of reads of different length (16-30 nt) mapping to dsRNAs targeting ATP synthase β (dsATP) or green fluorescent protein (dsGFP) in the six sRNA libraries analysed from the two groups of insects injected with either dsATP (Eva_ATP1, Eva_ATP2, Eva_ATP3) or dsGFP (Eva_GFP1, Eva_GFP2, Eva_GFP3).

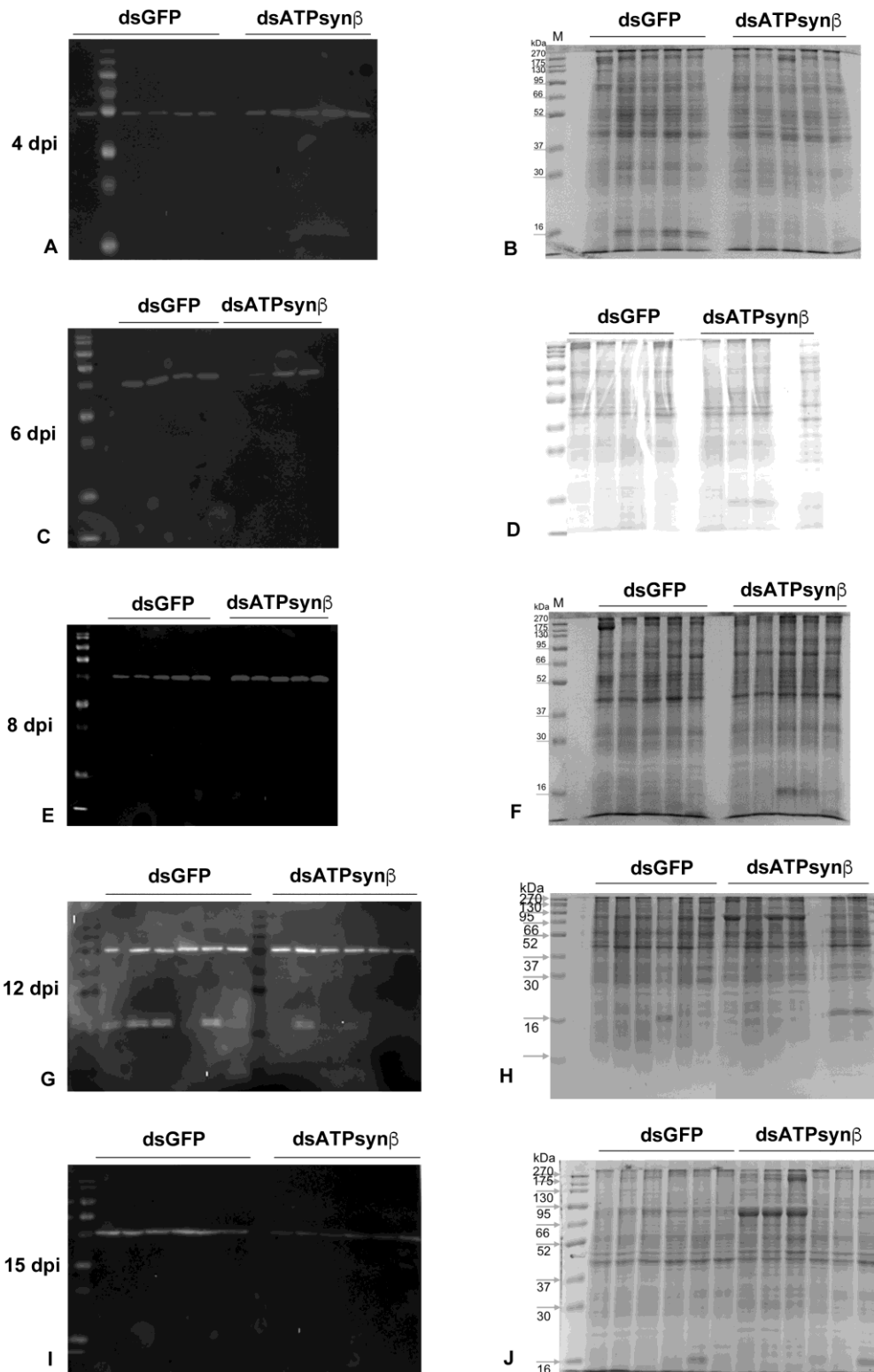
Reads mapping	Reads length	Library names					
		Eva_ATP1	Eva_ATP2	Eva_ATP3	Eva_GFP1	Eva_GFP2	Eva_GFP3
Mapping to dsATP	16	21071	33198	16810	31	430	140
	17	18519	28025	16425	41	384	146
	18	18305	32338	22263	33	352	150
	19	35983	63339	48217	26	621	291
	20	41256	79437	62671	22	280	149
	21	423242	830849	710122	68	420	339
	22	42734	68026	57132	39	340	178
	23	12293	11520	10636	13	223	145
	24	8385	5630	5925	15	197	163
	25	10718	6720	6084	24	300	241
	26	6732	3370	3367	15	185	155
	27	7646	3169	3419	22	320	208
	28	4269	1955	1867	6	82	67
	29	4477	1517	1560	6	106	68
	30	1532	457	618	1	53	39
Total dsATP mapped reads		657162	1169550	967116	362	4293	2479
Mapping to dsGFP	16	7	61	5	36400	23395	15696
	17	6	52	16	40724	26001	19222
	18	3	67	13	41780	27448	24382
	19	3	69	16	64683	43726	41178
	20	9	39	20	86864	60390	59237
	21	61	70	65	790116	525007	538637
	22	12	32	13	92659	60695	66046
	23	8	37	10	27650	18516	20514
	24	4	28	8	21753	14643	16880
	25	1	22	3	13859	8503	9623
	26	1	13	2	8915	5416	6723
	27	1	6	6	6502	4067	5236
	28	2	11	2	6002	4254	4871
	29	2	4	0	3829	2497	3008
	30	1	3	3	3309	2290	2833
Total dsGFP mapped reads		121	514	182	1245045	826848	834086
Total reads in library		22281562	19966749	22495258	27404377	22204066	28271348

Supplementary Table S4. Mean intensity/mm² of Western blots (WB) bands depicted in Figure 4 ± standard error of the mean (SEM) measured in *Euscelidius variegatus* insects, sampled at 15 days post injection of dsRNAs targeting ATP synthase β or green fluorescent protein (GFP). This parameter is the sum of the intensity of each pixel calculated by Quantity One 1-D Analysis Software (Bio-Rad) included in the band boundary manually defined.

Days post injection	Target of dsRNAs	Mean intensity/mm ² of WB bands ± SEM (N)
15	ATP synthase β	2.37E07 ± 2.75E06 (6)
	GFP	5.83E07 ± 7.17E06 (6)

Supplementary Table S5. Mean phytoplasma 16S RNAs/insect GAPDH transcript ± standard error of the mean (SEM) measured in *Euscelidius variegatus* insects, sampled at 22 days post injection of dsRNAs targeting ATP synthase β or green fluorescent protein (GFP) and at 7 days post acquisition of chrysanthemum yellows (CYp) or Flavescence dorée (FDp) phytoplasmas, as detailed in methodology depicted in Figure 1.

Target of dsRNAs	Phytoplasma acquired post dsRNA injection	Mean phytoplasma quantity ± SEM (N)
ATP synthase β	CYp	6.94E-03 ± 2.12E-03 (42)
GFP		3.74E-02 ± 1.03E-02 (43)
ATP synthase β	FDp	2.49E-02 ± 1.00E-02 (39)
GFP		1.01E-01 ± 2.68E-02 (36)



Supplementary Figure S1. Full length images of Western blots (WB) (left column, A, C, E, G, I) depicted in Figure 4, developed with anti-ATP synthase β antibody, and corresponding Coomassie stained SDS-polyacrylamide gels (right column, B, D, F, H, J), run in parallel with WB on the same *Euscelidius variegatus* insect samples (one insect sample per single lane), analysed at 4, 6, 8, 12, 15 days post injection (dpi) of dsRNAs targeting ATP synthase β (dsATPsyn β) or green fluorescent protein (dsGFP).