

Silencing of ATP synthase β impairs egg development in the leafhopper *Scaphoideus titanus*, vector of the phytoplasma associated with grapevine Flavescence dorée

Matteo Ripamonti¹, Luca Cerone^{1,2}, Simona Abbà¹, Marika Rossi¹, Sara Ottati^{1,2}, Sabrina Palmano¹, Cristina Marzachi¹ and Luciana Galetto^{1,*}

¹ Istituto per la Protezione Sostenibile Delle Piante, Consiglio Nazionale Delle Ricerche, IPSP-CNR, Strada delle Cacce 73, 10135 Torino, Italy; matteo.ripamonti@list.lu (M.R.); cerone_luca@libero.it (L.C.); simona.abba@ipsp.cnr.it (S.A.); marika.rossi@ipsp.cnr.it (M.R.); sara.ottati@ipsp.cnr.it (S.O.); sabrina.palmano@ipsp.cnr.it (S.P.); cristina.marzachi@ipsp.cnr.it (C.M.)

² Environmental Research and Innovation Department (ERIN), Luxembourg Institute of Science and Technology (LIST), 41 Rue du Brill, 4422 Luxembourg, Luxembourg

³ Dipartimento di Scienze Agrarie, Forestali ed Alimentari DISAFA, Università degli Studi di Torino, Largo Paolo Braccini 2, Grugliasco, 10095 Torino, Italy

* Correspondence: luciana.galetto@ipsp.cnr.it

Supplementary Tables

Supplementary Table S1. Over time expression of ATP synthase β transcript in *Scaphoideus titanus* following dsRNA injection. Mean \pm standard error of the mean (SEM), total number of samples (N), maximum/minimum and median of normalized relative expression of ATP synthase β transcript measured in *Scaphoideus titanus* sampled at 3, 6, 8, 10 and 14 days post injection of dsRNAs targeting green fluorescent protein (GFP) or ATP synthase β (St_dsATP1).

| Days post injection | Target of dsRNAs | ATP synthase β expression | | |
|---------------------|----------------------|---------------------------------|-------------|--------|
| | | Mean \pm SEM (N) | Max/Min | Median |
| 3 | GFP | 1.278 \pm 0.194 (5) | 1.928/0.796 | 1.250 |
| | ATP synthase β | 0.280 \pm 0.056 (5) | 0.477/0.125 | 0.258 |
| 6 | GFP | 1.387 \pm 0.149 (5) | 1.956/1.113 | 1.235 |
| | ATP synthase β | 0.280 \pm 0.038 (5) | 0.289/0.049 | 0.145 |
| 8 | GFP | 1.268 \pm 0.268 (5) | 2.110/0.656 | 1.028 |
| | ATP synthase β | 0.220 \pm 0.113 (4) | 0.557/0.081 | 0.120 |
| 10 | GFP | 1.185 \pm 0.072 (5) | 1.327/1.034 | 1.093 |
| | ATP synthase β | 0.060 \pm 0.009 (5) | 0.083/0.035 | 0.059 |
| 14 | GFP | 0.676 \pm 0.111 (4) | 0.952/0.408 | 0.672 |
| | ATP synthase β | 0.101 \pm 0.045 (5) | 0.272/0.022 | 0.080 |
| 20 | GFP | 1.140 \pm 0.299 (4) | 1.894/0.573 | 1.047 |
| | ATP synthase β | 0.025 \pm 0.007 (4) | 0.045/0.011 | 0.023 |

Supplementary Table S2. Expression of selected transcripts related with egg development in *Scaphoideus titanus* females following dsRNA injection. Mean \pm standard error of the mean (SEM), total number of samples (N), maximum/minimum and median of normalized relative expression of ATP synthase β , vitellogenin, hexamerin and cathepsin L transcripts measured in *Scaphoideus titanus* females (remaining parts of body after dissection of ovaries observed in Figure 3) sampled at 20 days post injection of dsRNAs targeting green fluorescent protein (GFP) or ATP synthase β (St_dsATP1).

| Target genes | Target of dsRNAs | Normalized relative quantities | | |
|----------------------|----------------------|--------------------------------|-------------|--------|
| | | Mean \pm SEM (N) | Max/Min | Median |
| ATP synthase β | GFP | 1.493 \pm 0.239 (5) | 2.203/0.738 | 1.491 |
| | ATP synthase β | 0.033 \pm 0.022 (7) | 0.168/0.005 | 0.013 |
| vitellogenin | GFP | 1.717 \pm 0.482 (5) | 3.098/0.450 | 1.280 |
| | ATP synthase β | 0.977 \pm 0.124 (9) | 1.721/0.459 | 0.991 |
| hexamerin | GFP | 0.441 \pm 0.169 (5) | 0.956/0.118 | 0.278 |
| | ATP synthase β | 3.273 \pm 1.302 (4) | 7.125/1.383 | 2.291 |
| cathepsin L | GFP | 1.608 \pm 0.424 (5) | 3.131/0.673 | 1.440 |
| | ATP synthase β | 0.609 \pm 0.096 (4) | 0.857/0.390 | 0.594 |

Supplementary Table S3. Over time expression of ATP synthase β transcript in *Euscelidius variegatus* following injection of different constructs of dsRNAs. Mean \pm standard error of the mean (SEM), total number of samples (N), maximum/minimum and median of normalized relative expression of ATP synthase β transcript measured in *Euscelidius variegatus* sampled at 7 and 15 days post injection of 80 and 8 ng per insect of dsRNAs targeting green fluorescent protein (GFP) or ATP synthase β of *E. variegatus* (Eva_dsATP1, Eva_dsATP2) and of *Scaphioideus titanus* (St_dsATP1 and St_dsATP2).

| Dose of dsRNAs (ng/insect) | Days post injection | Target of dsRNAs | ATP synthase β expression | | |
|-------------------------------|------------------------|------------------|---------------------------------|-------------|--------|
| | | | Mean \pm SEM (N) | Max/Min | Median |
| 80 | 7 | GFP | 1.727 \pm 0.256 (6) | 2.522/1.076 | 1.553 |
| | | Eva_dsATP1 | 0.017 \pm 0.002 (6) | 0.024/0.010 | 0.015 |
| | | Eva_dsATP2 | 0.016 \pm 0.004 (6) | 0.036/0.009 | 0.013 |
| | | St_dsATP1 | 0.061 \pm 0.013 (6) | 0.100/0.014 | 0.063 |
| | | St_dsATP2 | 0.065 \pm 0.023 (6) | 0.166/0.020 | 0.044 |
| | 15 | GFP | 1.159 \pm 0.102 (5) | 1.557/0.989 | 1.076 |
| | | Eva_dsATP1 | 0.017 \pm 0.006 (5) | 0.031/0.003 | 0.012 |
| | | Eva_dsATP2 | 0.015 \pm 0.001 (5) | 0.019/0.013 | 0.015 |
| | | St_dsATP1 | 0.014 \pm 0.004 (5) | 0.027/0.004 | 0.014 |
| | | St_dsATP2 | 0.060 \pm 0.037 (5) | 0.206/0.016 | 0.025 |
| 8 | 7 | GFP | 1.459 \pm 0.113 (6) | 1.977/1.199 | 1.406 |
| | | Eva_dsATP1 | 0.037 \pm 0.009 (5) | 0.065/0.017 | 0.031 |
| | | Eva_dsATP2 | 0.023 \pm 0.009 (6) | 0.066/0.008 | 0.019 |
| | | St_dsATP1 | 0.203 \pm 0.068 (6) | 0.512/0.054 | 0.175 |
| | | St_dsATP2 | 0.060 \pm 0.024 (5) | 0.138/0.016 | 0.029 |
| | 15 | GFP | 0.871 \pm 0.102 (6) | 1.326/0.624 | 0.805 |
| | | Eva_dsATP1 | 0.011 \pm 0.002 (5) | 0.016/0.005 | 0.011 |
| | | Eva_dsATP2 | 0.015 \pm 0.002 (7) | 0.023/0.009 | 0.014 |
| | | St_dsATP1 | 0.048 \pm 0.010 (6) | 0.088/0.020 | 0.045 |
| | | St_dsATP2 | 0.033 \pm 0.008 (6) | 0.072/0.015 | 0.026 |

Supplementary Table S4. List of primers used in this work.

| Application | Primer name | 5'-3' sequences | Organism/ target gene | Prod. size | qPCR efficiency | R ² | Melt peak |
|----------------------------|---|--|---|---------------|--------------------|----------------|--------------|
| dsRNA synthesis | St_T7ATPsynBeta ^f | <u>TAATACGACTCACTATAG</u> TGGCGCTGGTATACGGACAG | <i>S. titanus</i> / ATP synthase β | 480 nt | / | / | / |
| | St_T7ATPsynBeta ^r | TAATACGACTCACTATAGGGACGCCTCGAGCAATGTTG | | | | | |
| | St_T7ATPsynBeta ^f _2 [#] | <u>TAATACGACTCACTATAG</u> CAACGCTGTAACAAGGTCGG | <i>E. variegatus</i> / ATP synthase β | 195 nt | / | / | / |
| | St_T7ATPsynBeta ^r _2 [#] | TAATACGACTCACTATAGTCCACTACGGCACCAATCAC | | | | | |
| | Eva_T7ATPsynBeta ^f ¹ | <u>TAATACGACTCACTATAG</u> TACGGCCAGATGAACGAGCC | <i>E. variegatus</i> / ATP synthase β | 469 nt | / | / | / |
| | Eva_T7ATPsynBeta ^r ¹ | TAATACGACTCACTATAGGGACACCACGAGCAATGTTG | | | | | |
| | Eva_T7ATPsynBeta ^f _2 [#] | <u>TAATACGACTCACTATAG</u> CAACGCTGTAACAAGGTCTG | <i>S. titanus</i> / hexamerin | 195 nt | / | / | / |
| | Eva_T7ATPsynBeta ^r _2 [#] | TAATACGACTCACTATAGTCTACGACGGCACCGATCAC | | | | | |
| | T7GFP ^f ¹ | <u>TAATACGACTCACTATAG</u> CTTTTCACTGGAGTTGTCCC | GFP | 403 nt | / | / | / |
| | T7GFP ^r ¹ | TAATACGACTCACTATAGGTTTGTGTCCGAGAATGTTTC | | | | | |
| qPCR target genes | St_ATP β Fw622 ² | CGTTTCACTCAGGCTGGTTC | <i>S. titanus</i> / ATP synthase β | 171 nt | 85.4% | 0.999 | 82.5°C |
| | St_ATP β Rv792 ² | ATCATCAGCCGGCACATAGA | | | | | |
| | St_Vitel_F ² | AAGAGGAACATGCGCTCCTA | <i>S. titanus</i> / vitellogenin | 98 nt | 96.2% | 0.998 | 78.0°C |
| | St_Vitel_R ² | TTGCTGGGCAGGAACTATC | | | | | |
| | St_HEX_F710 ² | ACGCCAACCAGTACCAGAAC | <i>S. titanus</i> / hexamerin | 115 nt | 99.4% | 0.996 | 78.5°C |
| | St_HEX_R824 ² | GCAGGGTCTTCATCTGCTC | | | | | |
| | St_CathL473_F ² | CATCAAGGACCAGGGCCACTG | <i>S. titanus</i> / cathepsin L | 92 nt | 95.7% | 0.998 | 82.0°C |
| | St_CathL473_R ² | CCAGCTTTCCTGACTTGCGG | | | | | |
| | ATP β Fw622 ² | CGCTTTACTCAGGCTGGTTC | <i>E. variegatus</i> / ATP synthase β | 171 nt | 100% | 0.995 | 84.5°C |
| | ATP β Rv792 ² | GTCATCAGCTGGCACGTAGA | | | | | |
| qPCR reference genes | St_EF1- α _F215 ³ | CCATTGACATTGCCCTGTGG | <i>S. titanus</i> / elong. factor-1 α | 110 nt | 98.0% | 0.999 | 77.5°C |
| | St_EF1- α _R325 ³ | CCTGAGAAGTTCCAGTAATCATG | | | | | |
| | St_GST1_F257 ³ | CTAAGGATGCCCAGAAACGA | <i>S. titanus</i> / glut. transfer. | 113 nt | 94.5% | 0.998 | 79.0°C |
| | GST1_R369 ³ | TGGCGCTCCTCCAAACATCA | | | | | |
| | EF1- α _F215 ³ | CCATCGACATTGCCCTGTGG | <i>E. variegatus</i> / elong. factor-1 α | 111 nt | 87.9% | 0.997 | 78.5°C |
| | EF1- α _R325 ³ | CCTGTGAGGTTCCAGTGATCATG | | | | | |
| | GST1_F257 ³ | CCAAGGACCCCAAGAAGCGA | <i>E. variegatus</i> / glut. transfer. | 113 nt | 87.3% | 0.997 | 79.0°C |
| | GST1_R369 ³ | TGGCGCTCCTCCAAACATCA | | | | | |

T7 promoter sequence is underlined.

[#]Primers designed in this work.

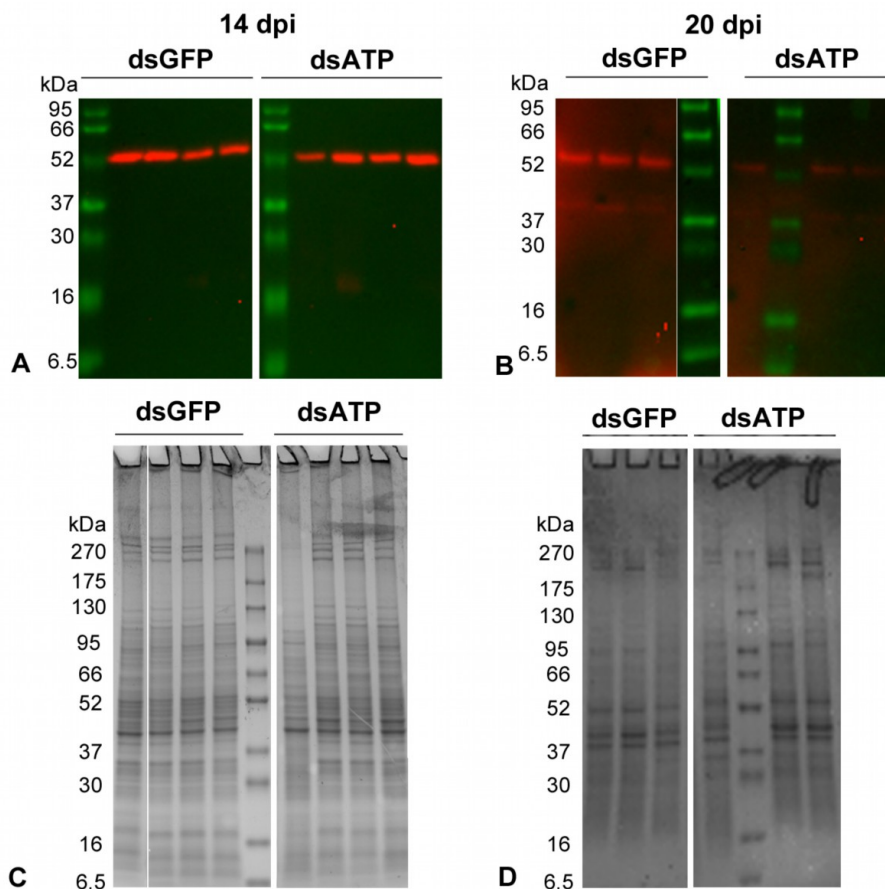
¹ Abbà et al., 2019

² Galetto et al., 2013

³ Galetto et al., 2018

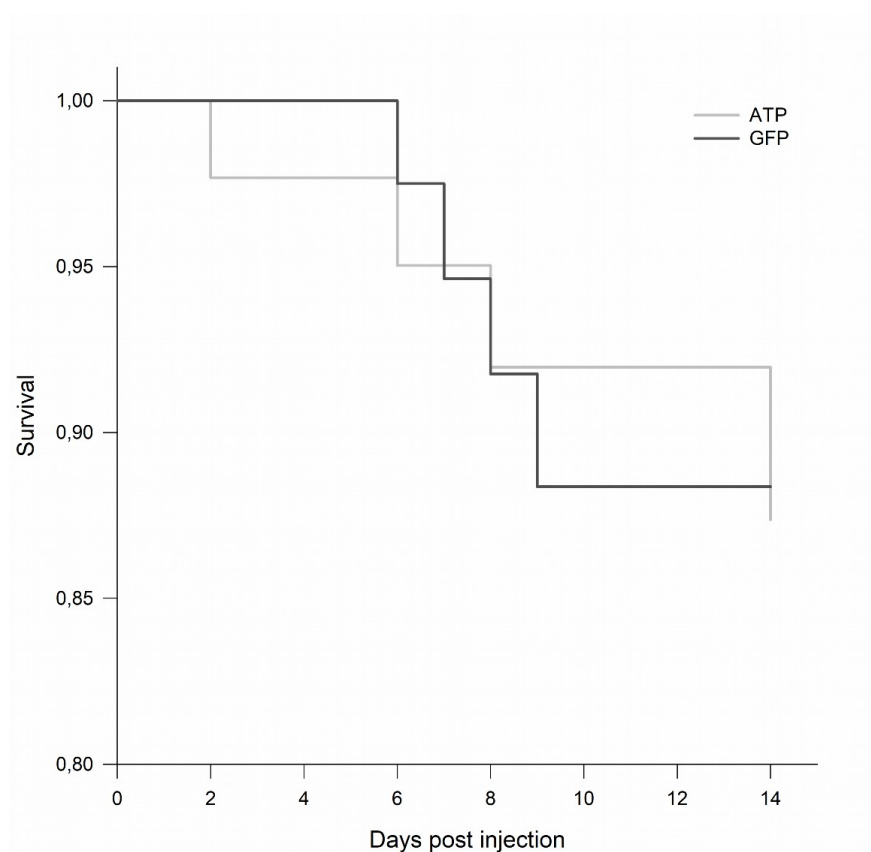
Supplementary Figures

Supplementary Figure S1



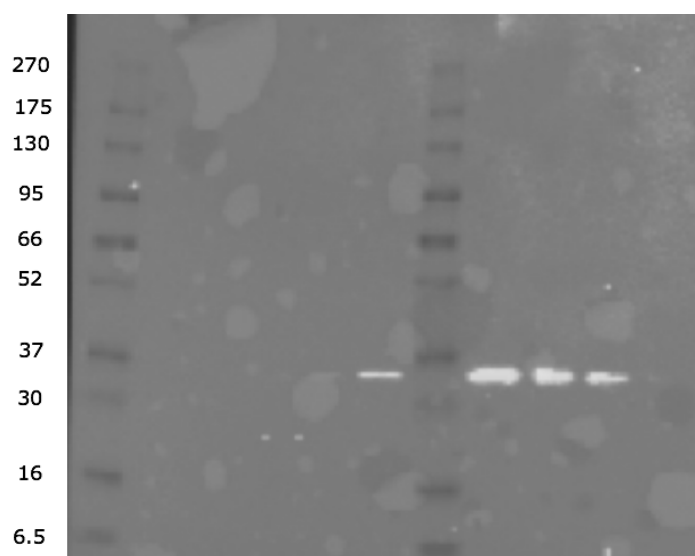
Supplementary Figure S1. Full length images of Western blots depicted in Figure 2 and corresponding SDS-PAGE. Full length images of Western blots (WB) (upper panels, A and B) depicted in Figure 2, developed with anti-ATP synthase β antibody, and corresponding Coomassie stained SDS-polyacrylamide gels (lower panels, C and D), run in parallel with WB on the same *Scaphoides titanus* insect samples (one insect sample per single lane), analyzed at 14 (panels A and C) and 20 (panels B and D) days post injection (dpi) of dsRNAs targeting green fluorescent protein (dsGFP) or ATP synthase β (dsATP), with construct St_dsATP1. The masses of protein standards migrated together with samples are indicated in kDa.

Supplementary Figure S2



Supplementary Figure S2. Survival rates of *Scaphoideus titanus* following dsRNA injection. Survival rates of 61 *Scaphoideus titanus* adults over a 14-day period after microinjection of 80 ng of dsRNAs targeting green fluorescent 62 protein (GFP) or ATP synthase β (ATP), with construct St_dsATP1.

Supplementary Figure S3



Supplementary Figure S3. Full lenght image of Western blots depicted in Figure 3. Full lenght image of Western blots depicted in Figure 3, developed with anti-cathepsin L primary antibody, on ovaries dissected from *Scaphoides titanus* females (ovaries from one insect per single lane) sampled at 20 days post injection of dsRNAs targeting green fluorescent protein (dsGFP) or ATP synthase β (dsATP), with construct St_dsATP1. The masses of protein standards migrated together with samples are indicated in kDa.