**Supplemental Legends**

**FIG S1** Nucleotide sequence alignment of the *iglA-D* containing region of *F.n.n.* FSC769 and other selected *Francisella* sp.: *F.n.o.* LADL--07-285A (GI: 564747871), *F. philomiragia* ATCC 25017 (GI: 167596226), *F. novicida* U112 (GI: 754269614), *F. holarctica* LVS (GI: 754265763) and *F.t.t.* Schu S4 (GI: 754282044). Pink background indicates nucleotide differences and dots represent identical residues between strains. Genes are labeled with yellow arrows behind the nucleotide sequence pointing in the coding direction and the gene order is *iglA, iglB, iglC* and *iglD*. *F.n.o*. has a premature stop codon 21 bp before the end of *iglA* compared to the other *Francisella* sp. analyzed.

**FIG S2** Representative dotplots of *Dictyostelium* wt cells infected with *F.n.n.* wt and Δ*iglC* (upper and middle panel) and *Dictyostelium* Δ*atg1* cells infected with *F.n.n.* wt (lower panel) at 1, 4, 24 and 48 hpi. Displayed is the sideward scatter (SSC) plotted as a function of green fluorescence (FL-1). After enhancement of the endogenous *F.n.n.* GFP signal with an AlexaFluor488-coupled antibody we observe two separate populations of weakly (non-infected) and strongly fluorescent (infected) cells.

**FIG S3** Replication of *F.n.n.* in zebrafish is dependent on IglC

(A) Site of microinjection (white circle) and observation area of bacterial growth using fluorescence microscopy (black rectangle). (B-G) Representative micrographs show the fluorescent signal of *F.n.n.* wt (B, C), *F.n.n.* Δ*iglC* (D, E) and PBS as a negative control (F, G) at 6 dpi. Displayed are the green channel (GFP) and an overlay with the corresponding bright field image of the zebrafish tail region. (H) Bacterial replication of *F.n.n.* wt (black), *F.n.n.* Δ*iglC* (white) and *F.n.n. iglC*+ (grey) measured by qPCR at 3 and 7 dpi (n=3). In the *F.n.n.* Δ*iglC* infected group (6 dpi), a few of the embryos displayed a substantial amount of green fluorescent granulas in parts of the yolk sac. This could suggest that the *F.n.n.* Δ*iglC* mutant succeeds in some individuals, however selected individual responses of zebrafish embryos to bacterial infections were observed before (91). This different degree of success is reflected in the gDNA samples, where one Δ*iglC* sample (consisting of 3 embryos each) contained a much higher quantity of *F.n.n.* genomes than the others 7 dpi.

**FIG S4** Additional ultrastructural analysis of intracellular *F.n.n*. wt samples processed by chemical fixation and room temperature dehydration (A, B) or HPF and freeze substitution (C, D). (A, B) Ultrastructural analysis of phagosomal (A) and cytosolic (B) *F.n.n.* after chemical fixation at 2 and 4 hpi, respectively. Cytosolic as well as phagosomal bacteria are surrounded with the electron-translucent area, which indicates fixation artefacts. (C, D) Overview (C) and high magnification (D) showing formerly cytosolic *F.n.n*. wt at 1 hpi possibly contained within an autophagosome as indicated by the limiting autophagic-like membrane and the ribosomes in the lumen. Bacteria are surrounded with an electron pale area indicating the presence of weakly contrasted material, which was a characteristic appearance of cytosolic bacteria after HPF and freeze substitution. No binding of various lectins (wheat germ agglutinin, concanavalin A, ricinus agglutinin I, *Erythrina cristagalli* lectin, *Maackia amurensis* Lectin I or *Maclura pomifera* lectin) was observed indicating no enrichment in mannose, glucose, N-acetylglucosamine, galactose, N-acetylgalactoseamine or sialic acid components. Asterisks: *F.n.n.*, arrows: *F.n.n.*-enclosing membrane, white arrowheads: electron-translucent area characteristic of chemically fixed bacteria, black arrowheads: electron-pale area characteristic for HPF. Scale bars: 500 nm (A, B, D), 2 µm (C).

**Fig S5** Visualization of the rupture of the phagosomal membrane by electron and fluorescence microscopy. (A, B) Electron microscopy following HPF and freeze substitution reveals rupturing phagosomal membrane in *Dictyostelium* cells infected with *F.n.n.* wt at 1 hpi. Asterisks: *F.n.n.*, arrowheads: broken membrane. (C, D) Confocal fluorescence microscopy shows the broken p80-positive compartment (green) and escaping *F.n.n.* wt (red) at 4 hpi. The inlet displays the green channel (p80) of the region of interest (white square). Scale bars: 500nm (A, B), 5 µm (C, D).

**FIG S6** Phagosomal maturation and ubiquitination in *Dictyostelium* Δ*atg1* cells

(A-C) Association rates of *F.n.n.* wt with p80 (A), VatA (B) and ubiquitin (C) in *Dictyostelium* Δ*atg1* cells over 48 hpi. Data of 3 independent experiments (mean±SEM) was collected for each association study and a minimum of 100 bacteria was counted at each timepoint. (D) Representative micrographs of accumulated, ubiquitinated (green) *F.n.n.* wt (red) in *Dictyostelium* Δ*atg1* cells at 48 hpi. The margin of the cell is marked with a dashed line and the inlet shows only ubiquitin in the green channel. Scale bar: 5 µm.

**Movie S1** Live cell microscopy of *Dictyostelium* Lifeact-RFP (red) infected with *F.n.n.* wt expressing GFP (green) at 19 hpi. The inlet displays the red channel (actin) of the region of interest (white square). Scale bar: 5 µm.

**Movie S2** Live cell microscopy of *Dictyostelium* Lifeact-RFP (red) infected with *F.n.n.* Δ*iglC* expressing GFP (green) at 2 hpi. The inlet displays the red channel (actin) of the region of interest (white square). Scale bar: 5 µm.

**Cryofixation, chemical fixation and Transmission Electron Microscopy**

Cellulose capillary tubes (a kind gift by Heinz Schwarz) with an inner diameter of 200 µm were filled with a concentrated suspension of infected cells by capillary action (Hohenberg *et al.*, 1994), cut into short segments and transferred into aluminium planchettes (with a 6 mm diameter and a 150 µm recess), filled with 1-hexadecene (Merck Millipore, Darmstadt, Germany). Samples were cryofixed in an EM HPM100 high-pressure freezing machine (Leica Microsystems, Vienna, Austria) (Kaech *et al.*, 2014). Freeze substitution (Balzers FSU 010; Bal-Tec, Balzers, Liechtenstein) was performed as described (Leonidova *et al.*, 2014) in acetone containing 1% osmium tetroxide for 8 h at -90°C, 6 h at -60°C and 4 h at -40°C. Afterwards samples were incubated for 1 h on ice, washed with acetone and incubated with 1% uranyl acetate in acetone for 1 h on ice. After washing samples were then progressively infiltrated with epon resin (Fluka, St. Louis, Missouri, USA) (15, 33, 50, 80, 100%) over two days at room temperature or they were first incubated with 0.5% glutaraldehyde (GA) for 30 min at 4°C, washed and then infiltrated with LR White resin (Electron Microscopic Sciences, Hatfield, PA, USA) (50, 100%) over 10 hours. Eventually samples were polymerized overnight at 70°C. Around 70 nm sections were cut using an ultra 45° diamond knife (Diatome, Biel, Switzerland). Sections were examined without additional contrasting using a CM100 transmission electron microscope (Philips, Eidhoven, Netherlands). The images were recorded digitally with a Quemesa TEM CCD camera and iTEM software v 5.1 (both Olympus Soft Imaging Solutions, Münster, Germany).

The embedding of chemically fixed samples was performed by the EM facility at the department of Biosciences, University of Oslo. Infected, attached cells were fixed in a solution of 2% GA and 1% PFA in HL5c for 2 h at RT and washed with sterile PBS. Subsequently, 2% GA/sodium cacodylate (0.1 M) was added before the fixed cells were scraped off the well bottom with a cell scraper and stored at 4°C. The cell pellets were subsequently washed 2 x 10 min with 0.1 M sodium cacodylate buffer, then incubated for 1 h in darkness with the aqueous solution of 2% $OsO_4$ and 1.5% $K_3Fe(CN)_6$, washed 2 x 10 min in $dH_2O$ and stained with 1.5% uranylacetate for 30 min. Samples were dehydrated in 70, 80, 90, 96% EtOH for 10 min in each concentration before 4 x 15 min in absolute EtOH. The pellets were resuspended in 1:1 epon/propylene oxide and rotated O/N with open lids before Epon polymerization at 60°C for 3 days. Sections were prepared and examined as described above for cryofixed samples.


Hohenberg, H., Mannweiler, K. and Muller, M. (1994). High-pressure freezing of cell suspensions in cellulose capillary tubes. *Journal of microscopy* **175,** 34-43.

Kaech, A. and Ziegler, U. (2014). High-pressure freezing: current state and future prospects. *Methods in molecular biology* **1117,** 151-171.

Leonidova, A., Pierroz, V., Rubbiani, R., Lan, Y.J., Schmitz, A.G., Kaech, A.*, et al.* (2014). Photo-induced uncaging of a specific Re(I) organometallic complex in living cells. *Chem Sci* **5,** 4044-4056.

```
F.n.n. FSC769              ATGGCGAAAAATAAGATTCCAAATTCAAGGTTGATGATAAATTATGAAACTAAAGTGGAT 60
F.n.o. LADL--07-285A       .....A.............................A....................T..... 60
F. philomiragia ATCC 25017 .....A.......................................C........G..... 60
F. novicida U112           .....A........A..C....................................T..T.. 60
F.t. holarctica LVS        .....A........A..C....................................T..T.. 60
F.t.t. Schu S4             .....A........A..C....................................T..T.. 60
Consensus                  ATGGCAAAAAATAANATNCCAAATTCAAGGTTGATGATAAATTATGAAACTAATGTNGAT

F.n.n. FSC769              GGGGTTCTAAAGAAAAAAGAATTGCCTTACAGAGTTTTAGTTGTTGGTGATTTGTCGAAA 120
F.n.o. LADL--07-285A       ..A...................A.........C......A.................... 120
F. philomiragia ATCC 25017 ..A...................A...............G..A..............A..G 120
F. novicida U112           ..T..CT.............GC.A...........CC..........C.....A..A... 120
F.t. holarctica LVS        ..T..CT.............GC.A...........CC..........C.....A..A... 120
F.t.t. Schu S4             ..T..CT.............GC.A...........CC..........C.....A..A... 120
Consensus                  GGTGTNNTAAAGAAAAAAGANNTACCTTACAGAGTNNTAGTTGTTGGNGATTTNTCAAAA

F.n.n. FSC769              GGAAGATCCGTAGATGCTAAAAAAGAGTTTATTGATAGAGCTGTTAGACGAGTAAATAAT 180
F.n.o. LADL--07-285A       ........G.......................C............................ 180
F. philomiragia ATCC 25017 ........G........A.............C.........G.................. 180
F. novicida U112           ........T..G.....A...........CGCA.......AG..C...A........... 180
F.t. holarctica LVS        ........T..G.....A...........CGCAT......AG..C...A........... 180
F.t.t. Schu S4             ........T..G.....A...........CGCA.......AG..C...A........... 180
Consensus                  GGAAGATCTGTNGATGCAAAAAAAGAGTTNNCNGATAGAGNGGTNAGANGAGTAAATAAT

F.n.n. FSC769              GGTGTTGATAGAGCCTTAGAAGATATGAATATATCTTTTGACTTTGAAGCTCCGAACTTT 240
F.n.o. LADL--07-285A       .........................................T........A..A..T..C 240
F. philomiragia ATCC 25017 .....................................................A...... 240
F. novicida U112           ...........G.TT........G.................T.....G..A..A...... 240
F.t. holarctica LVS        ...........G.TT........G.................T.....G..A..A...... 240
F.t.t. Schu S4             ...........G.TT........G.................T.....G..A..A...... 240
Consensus                  GGTGTTGATAGNGNNTTAGAAGANATGAATATATCTTTTGATTTTGANGCACCAAACTTT

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F.n.o. LADL--07-285A       ..C..T..A..C...G.C...T...................T.................. 300
F. philomiragia ATCC 25017 ........A................................................... 300
F. novicida U112           .....T..A............T...................TG.......C......... 300
F.t. holarctica LVS        .....T..A............T...................TG.......C......... 300
F.t.t. Schu S4             .....T..A....G.......T...................TG.......C......... 300
Consensus                  GTTTCTAAAGATCCTAGTAATTTAAAAGTTAATTATAGAATTNAAAGTGTNAAAGATTTT

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F.n.o. LADL--07-285A       .GG.................G........T..G..TA....CT.G..T...........A 360
F. philomiragia ATCC 25017 ....................................A....................... 360
F. novicida U112           .G...........................T......A....G..G..T............ 360
F.t. holarctica LVS        .G...........................T......A....G..G..T............ 360
F.t.t. Schu S4             .G...........................T......A....G..G..T............ 360
Consensus                  AGACCTGATGCTGTTGCTAAAAAAGTTCCTGAAATCAGAGCGCTGCTTGAAATGAAAGAG

F.n.n. FSC769              ATATTAGCATCTTTTGCTAAGAATATTGAAAATAATCGCAATCTTAAAAAGACTATAGAT 420
F.n.o. LADL--07-285A       .....................G................T..................... 420
F. philomiragia ATCC 25017 .....................G...................................... 420
F. novicida U112           ...........G.........G.C..............T.....C..G..A..C...... 420
F.t. holarctica LVS        ...........C.........G.C..............T.....C..G..A..C...... 420
F.t.t. Schu S4             ...........C.........G.C..............T.....C..G..A..C...... 420
Consensus                  ATATTAGCATCTTTTGCTAAGGANATTGAAAATAATCGTAATCTNAANAANACNATAGAT

F.n.n. FSC769              ATGATTTTTTCAGATAATAATGAGCTAGAAGCTTTAAAGAGTAAGATTCCTACTTTAACA 480
F.n.o. LADL--07-285A       ...................................................G........ 480
F. philomiragia ATCC 25017 ...................................................G........ 480
F. novicida U112           ................G...C..AT.....T.A..................G....G... 480
F.t. holarctica LVS        ................G...C..AT.....T.A..................G....G... 480
F.t.t. Schu S4             ................G...C..AT.....T.A..................G....G... 480
Consensus                  ATGATTTTTTCAGATANTAANGANNTAGAANCNTTAAAGAGTAAGATTCCTGCTTTNACA
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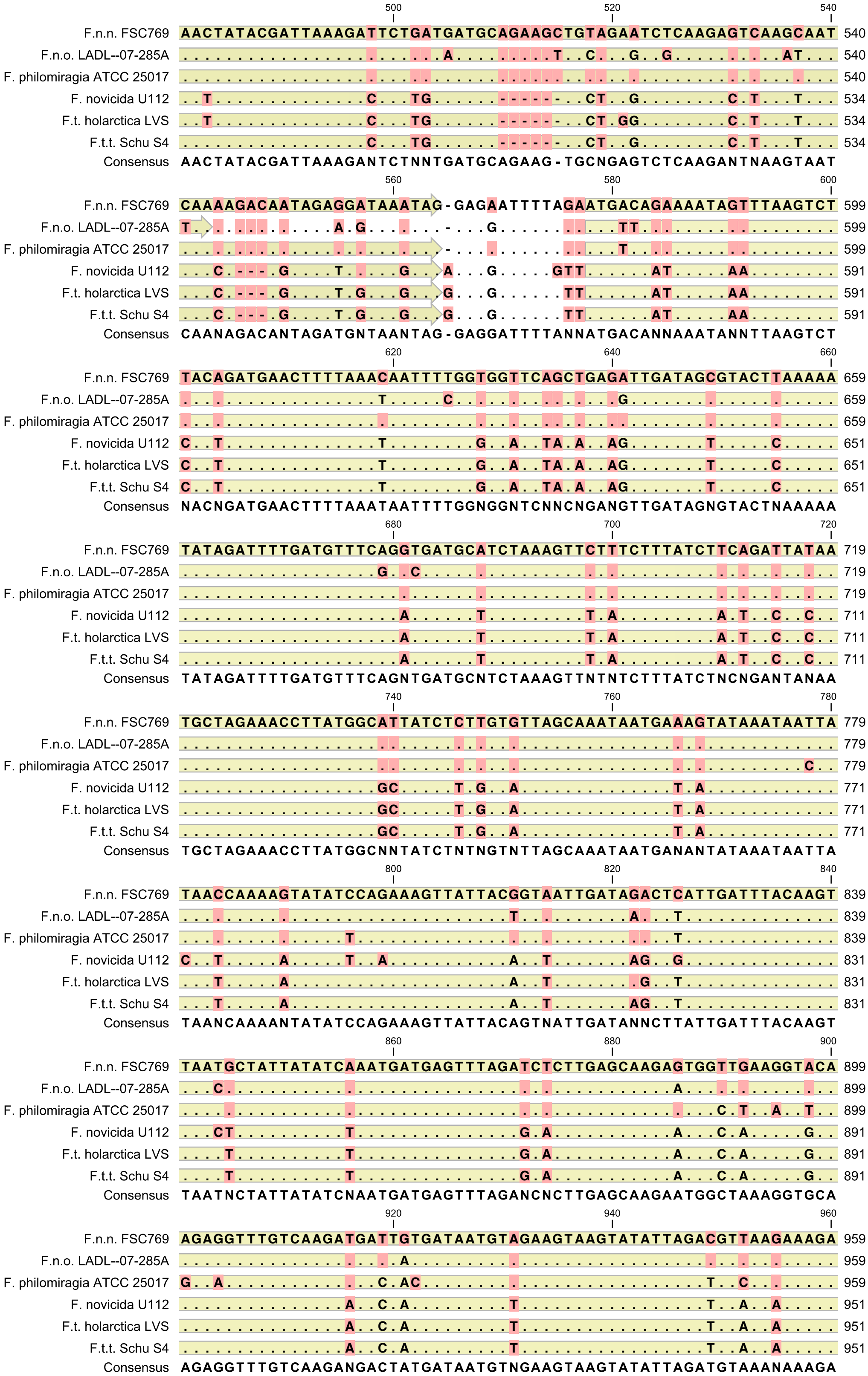
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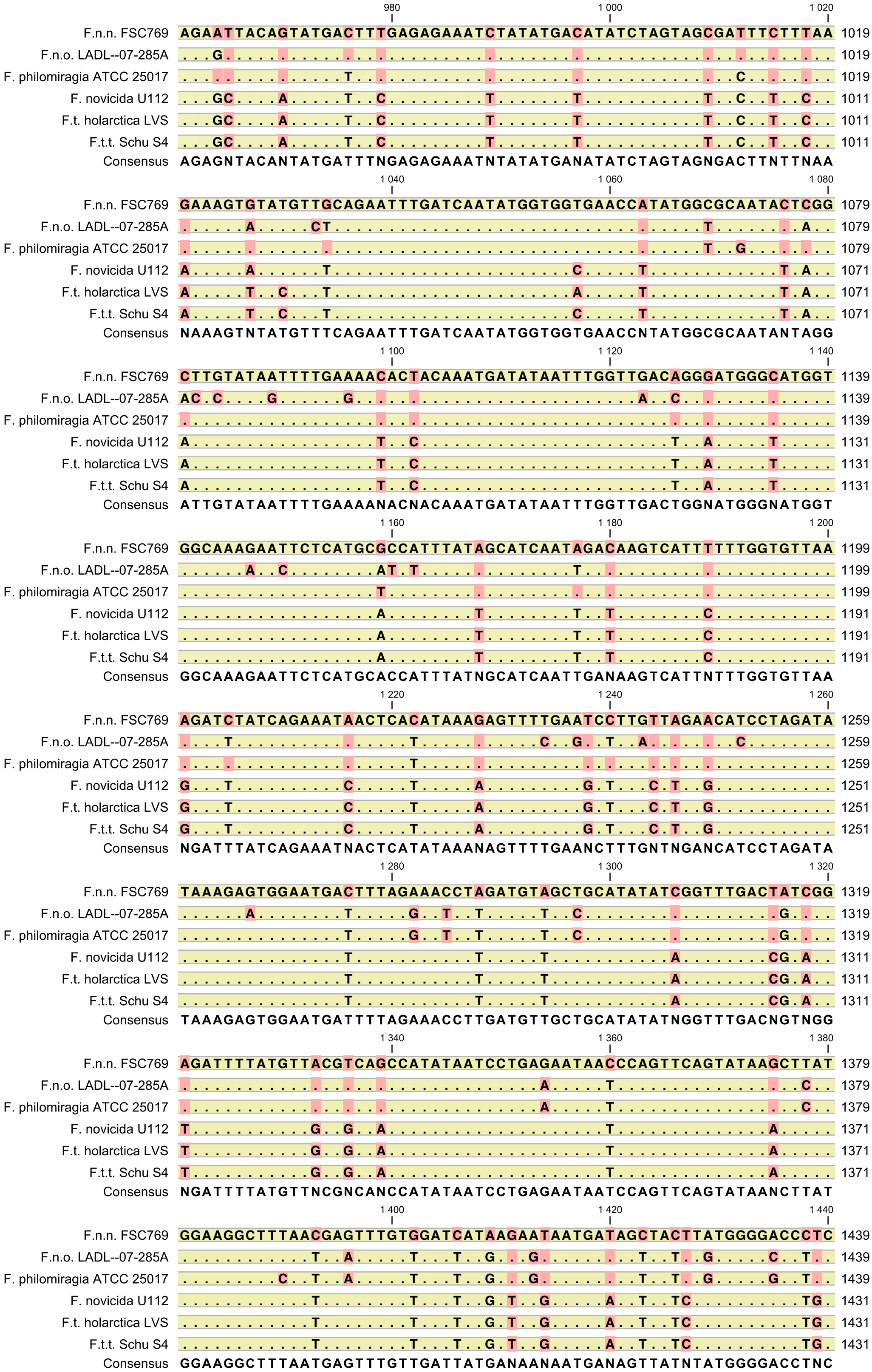
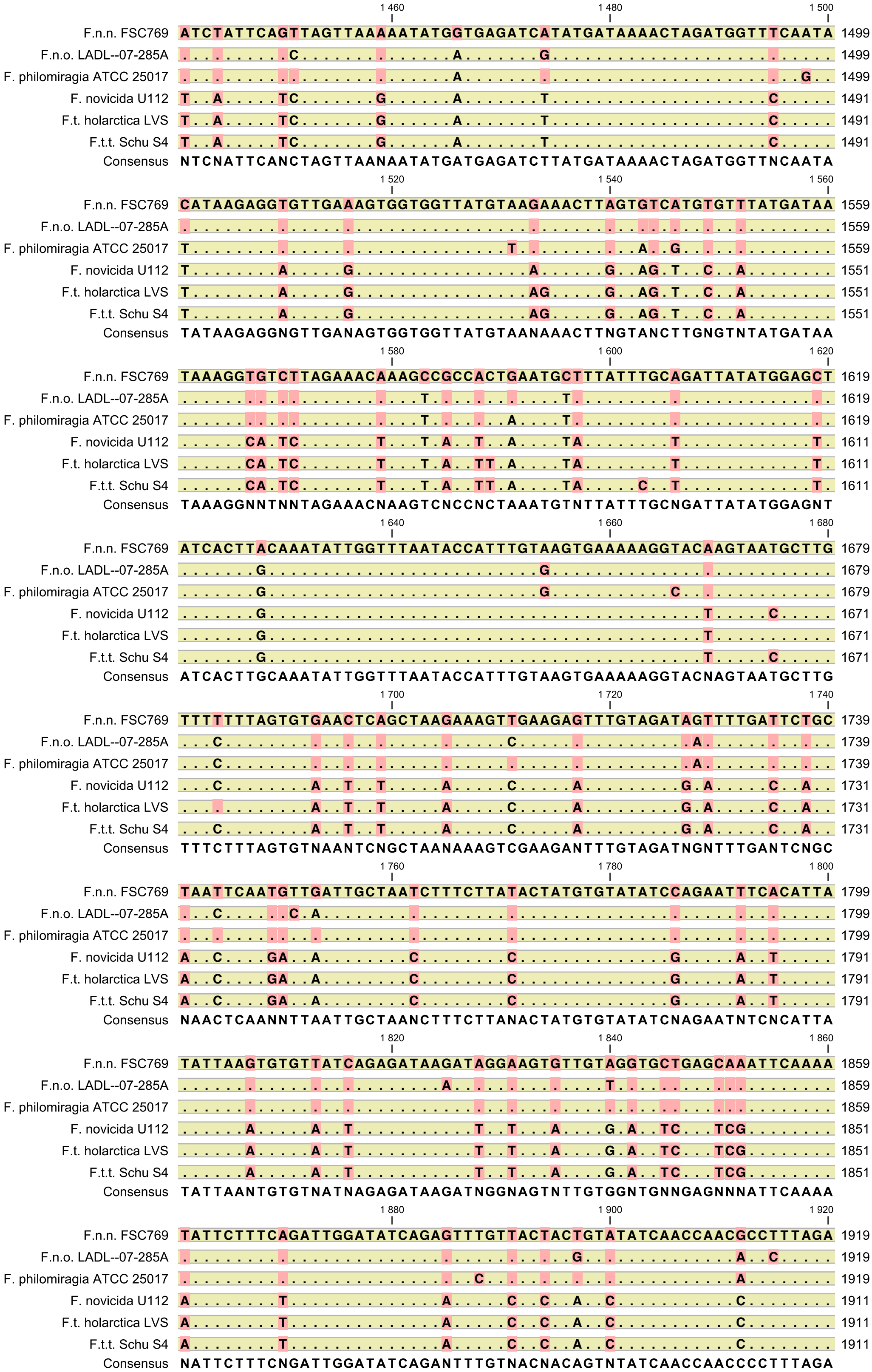
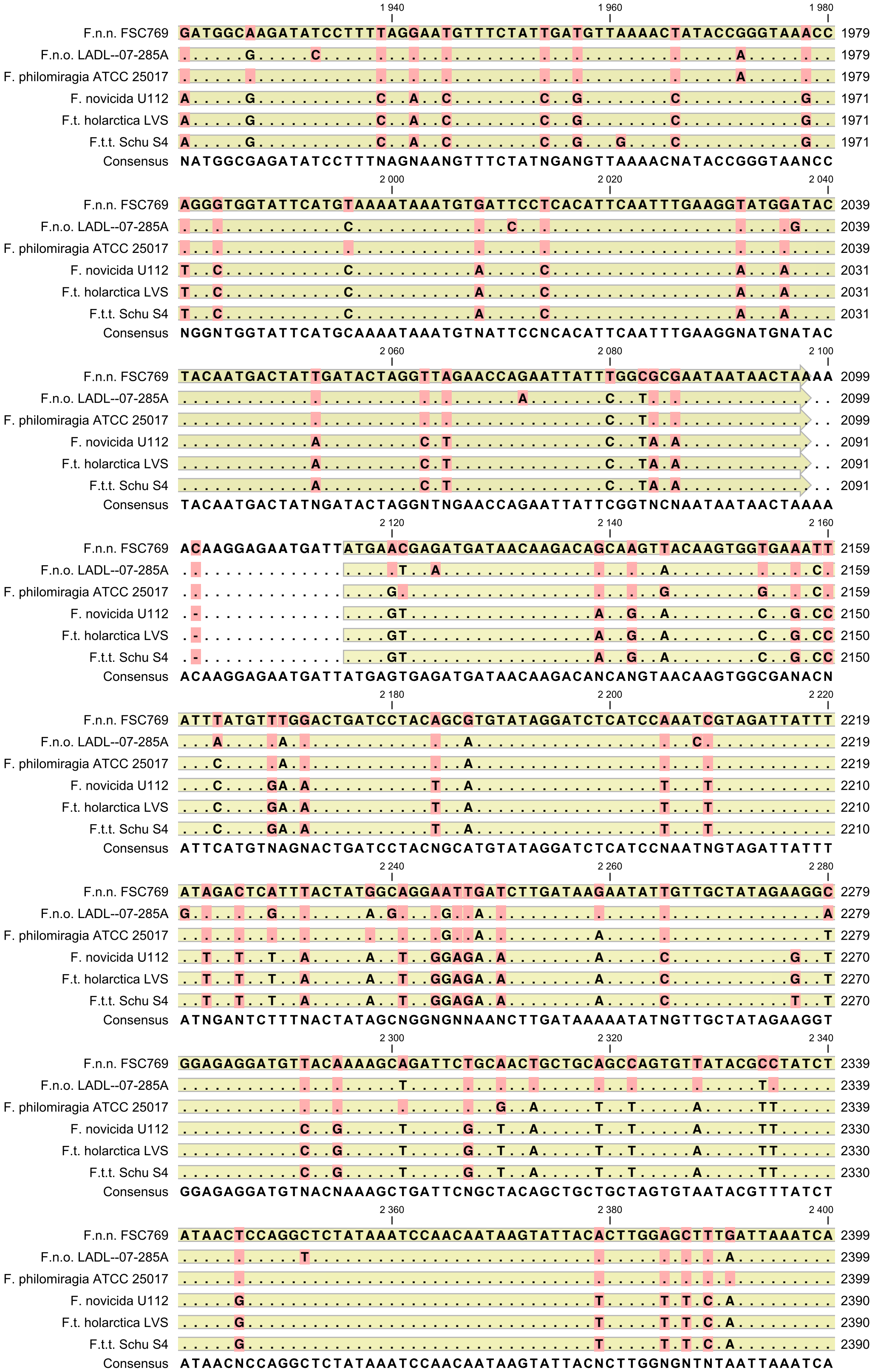
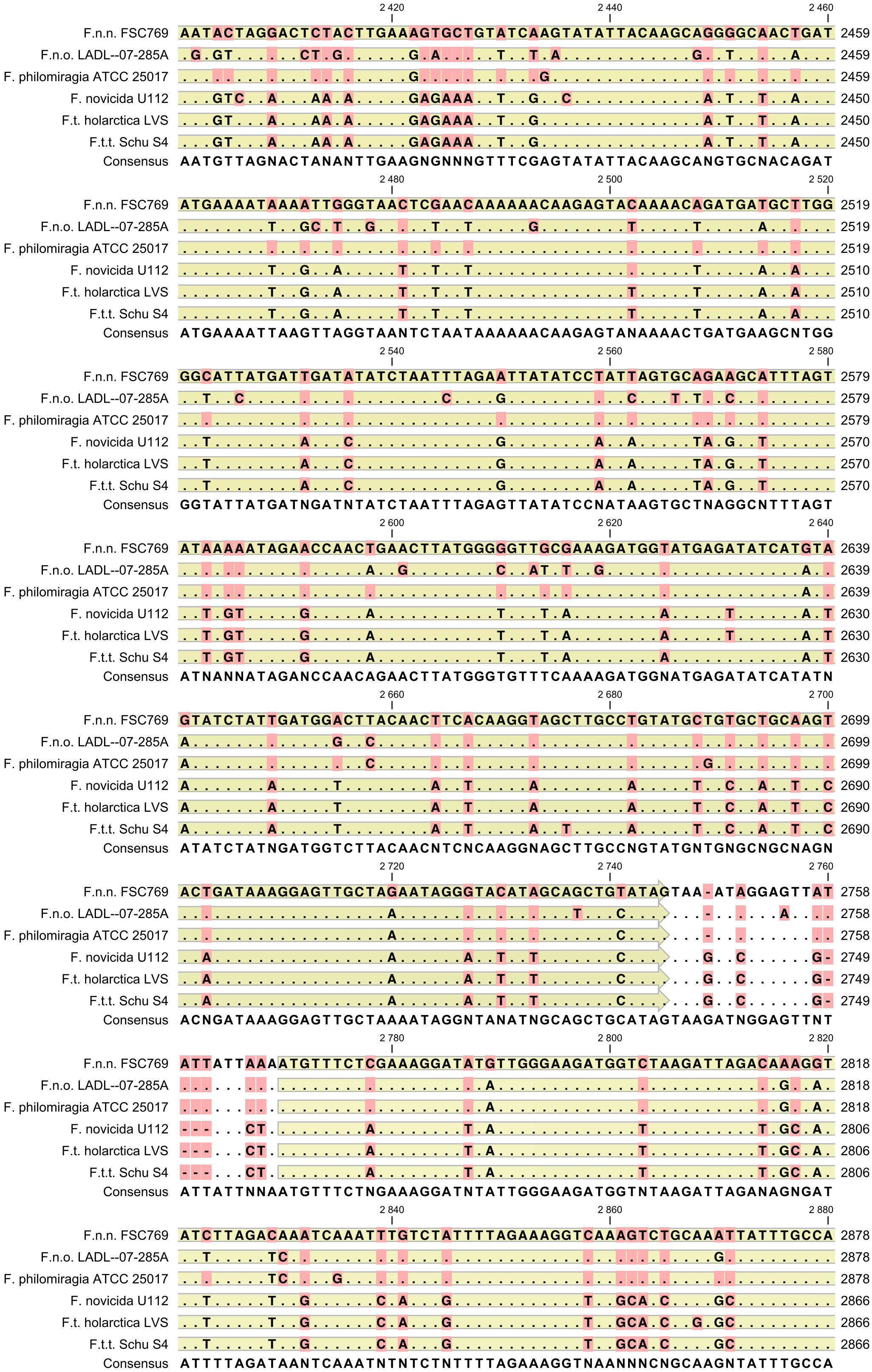
F.n.n. FSC769 CAAAAGACAATAGAGGATAAATAG-GAGAATTTTAGAATGACAGAAATAGTTTAAGTCT 599
Consensus CAANAGACANTAGATGNTAANTAG-GAGGATTTTANNATGACANNAAATANNTTAAGTCT
F.n.n. FSC769 TACAGATGAACTTTTAAACAATTTTGGTGGTTCAGCTGAGATTGATAGCGTACTTAAAAA 659
Consensus NACNGATGAACTTTTAAATAATTTTGGNGGNTCNNCNGANGTTGATAGNGTACTNAAAAA
F.n.n. FSC769 TATAGATTTTGATGTTTCAGGTGATGCATCTAAAGTTCTTTCTTTATCTTCAGATTATAA 719
Consensus TATAGATTTTGATGTTTCAGNTGATGCNTCTAAAGTTNTNTCTTTATCTNCNGANTANAA
F.n.n. FSC769 TGCTAGAAACCTTATGGCATTATCTCTTGTGTTAGCAAATAATGAAAGTATAAATAATTA 779
Consensus TGCTAGAAACCTTATGGCNNTATCTNTNGTNTTAGCAAATAATGANANTATAAATAATTA
F.n.n. FSC769 TAACCAAAAGTATATCCAGAAAGTTATTACGGTAATTGATAGACTCATTGATTTACAAGT 839
Consensus TAANCAAAANTATATCCAGAAAGTTATTACAGTNATTGATANNCTTATTGATTTACAAGT
F.n.n. FSC769 TAATGCTATTATATCAAATGATGAGTTTAGATCTCTTGAGCAAGAGTGGTTGAAGGTACA 899
Consensus TAATNCTATTATATCNAATGATGAGTTTAGANCNCTTGAGCAAGAATGGCTAAAGGTGCA
F.n.n. FSC769 AGAGGTTTGTCAAGATGATTGTGATAATGTAGAAGTAAGTATATTAGACGTTAAGAAAGA 959
Consensus AGAGGTTTGTCAAGANGACTATGATAATGTNGAAGTAAGTATATTAGATGTAAANAAAGA


F.n.n. FSC769
F.n.o. LADL--07-285A
F. philomiragia ATCC 25017
F. novicida U112
F.t. holarctica LVS
F.t.t. Schu S4
Consensus






F.n.n. FSC769
F.n.o. LADL--07-285A
F. philomiragia ATCC 25017
F. novicida U112
F.t. holarctica LVS
F.t.t. Schu S4
Consensus
2 420
2 440
2 460
AATACTAGGACTCTACTTGAAAGTGCTGTATCAAGTATATTACAAGCAGGGGCAACTGAT 2459
AATGTTAGNACTANANTTGAAGNGNNNGTTTCGAGTATATTACAAGCANGTGCNACAGAT
2 480
2 500
2 520
ATGAAAATAAAATTGGGTAACTCGAACAAAAAACAAGAGTACAAAACAGATGATGCTTGG 2519
ATGAAAATTAAGTTAGGTAATCTAATAAAAAACAAGAGTANAAAACTGATGAAGCNTGG
2 540
2 560
2 580
GGCATTATGATTGATATATCTAATTTAGAATTATATCCTATTAGTGCAGAAGCATTTAGT 2579
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2 600
2 620
2 640
ATAAAAATAGAACCAACTGAACTTATGGGGGTTGCGAAAGATGGTATGAGATATCATGTA 2639
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2 660
2 680
2 700
GTATCTATTGATGGACTTACAACTTCACAAGGTAGCTTGCCTGTATGCTGTGCTGCAAGT 2699
ATATCTATNGATGGTCTTACAACNTCNCAAGGNAGCTTGCCNGTATGNTGNGCNGCNAGN
2 720
2 740
2 760
ACTGATAAAGGAGTTGCTAGAATAGGGTACATAGCAGCTGTATAGTAA-ATAGGAGTTAT 2758
ACNGATAAAGGAGTTGCTAAAATAGGNTANATNGCAGCTGCATAGTAAGATNGGAGTTNT
2 780
2 800
2 820
ATTATTAAAATGTTTCTCGAAAGGATATGTTGGGAAGATGGTCTAAGATTAGACAAAGGT 2818
ATTATTNNAATGTTTCTNGAAAGGATNTATTGGGAAGATGGTNTAAGATTAGANAGNGAT
2 840
2 860
2 880
ATCTTAGACAAATCAAATTTGTCTATTTTAGAAAGGTCAAAGTCTGCAAATTATTTGCCA 2878
ATTTTAGATAANTCAAATNTNTCTNTTTTAGAAAGGTNAANNNCNGCAAGNTATTTGCCA

```
                                              2 900                         2 920                         2 940
F.n.n. FSC769              GCTAATCTTAATAAAGGTATCGTTAGTTTCGATTTAGATATGGAGAGTTTGCAGACGGGA 2938
F.n.o. LADL--07-285A       ..A.................TC...................A.................. 2938
F. philomiragia ATCC 25017 .........................................A..............T... 2938
F. novicida U112           ..............G..A........C..T.........G.T..A...........A..T 2926
F.t. holarctica LVS        ..............G..A........C..T.........G.T..A...........A..T 2926
F.t.t. Schu S4             ..............G..A........C..T.........G.T..A...........A..T 2926
Consensus                  GCTAATCTTAATAANGGNATCGTTAGNTTNGATTTAGATNTTGANAGTTTGCAGACAGGN

                                              2 960                         2 980                         3 000
F.n.n. FSC769              CTTATTCTAATAAAAGATCTTGTATTGTACTTAGATGAAAAAACATTTATTTTTTATGAT 2998
F.n.o. LADL--07-285A       ......A..................................................... 2998
F. philomiragia ATCC 25017 .........G...........A...................................... 2998
F. novicida U112           .....C..T............AA.................AT...G........... 2986
F.t. holarctica LVS        .....C..T............AA.................AT...G........... 2986
F.t.t. Schu S4             .....C..T............AA.................AT...G........... 2986
Consensus                  CTTATNCTNATAAAAGATCTTANATTGTACTTAGATGAAAAAANNTTTNTTTTTTATGAT

                                              3 020                         3 040                         3 060
F.n.n. FSC769              AAATGCTATCCTCTGTCTTTGCAGGTAATGACAGATGAGTTAACTAATGATATCCCTCTA 3058
F.n.o. LADL--07-285A       ..............A.........A..G................................ 3058
F. philomiragia ATCC 25017 ..............A............................................. 3058
F. novicida U112           ..G.CT.....GT.A.....A..AA.......T...A......G.G....A..A..CT.. 3046
F.t. holarctica LVS        ..G.CT.....GT.A.....A..AA.......T...A......G.G....A.....CT.. 3046
F.t.t. Schu S4             ..G.CT.....GT.A.....A..AA.......T...A......G.G....A.....CT.. 3046
Consensus                  AANTNNTATCCNNTATCTTTNCANATAATGACNGATNAGTTAANTNATGANATCCCNNTA

                                              3 080                         3 100                         3 120
F.n.n. FSC769              TTCTTGAATGTTAAGGAGAAGATAGTTGAAAAAGAAGGTGTTAAGTACATATATAATCAA 3118
F.n.o. LADL--07-285A       ..............T....................................C........ 3118
F. philomiragia ATCC 25017 .....A...................................................... 3118
F. novicida U112           ..TC.....A.C.GA.....AG..A........A.T..G.....A..T..C......... 3106
F.t. holarctica LVS        ..TC.....A.C.GA.....AG..A........A.T..GA....A..T..C......... 3106
F.t.t. Schu S4             ..TC.....A.C.GA.....AG..A........A.T..GA....A..T..C......... 3106
Consensus                  TTNNTGAATNTNANAGAGAANNTANTTGAAAAANANGGNGTTAANTANATNTATAATCAA

                                              3 140                         3 160                         3 180
F.n.n. FSC769              CTATCACTTTCATTAGAGTATGATTATAGTGTTAAGTATAGTACGCAAATTGCATTATTT 3178
F.n.o. LADL--07-285A       ..................C......................................... 3178
F. philomiragia ATCC 25017 ............................................................ 3178
F. novicida U112           T.G...T.A.........C..AGC...G..T....AC....C.TC............... 3166
F.t. holarctica LVS        T.G...T.A.........C..AGC...G..T....AC....C.TC............... 3166
F.t.t. Schu S4             T.G...T.A.........C..AGC...G..T....AC....C.TC............... 3166
Consensus                  NTNTCANTNTCATTAGAGCATNNNTATNGTNTTAANNATAGNANNCAAATTGCATTATTT

                                              3 200                         3 220                         3 240
F.n.n. FSC769              AAGTTAGATAGAGGCAAGTTAGTATCAGATACATATGATTTTCCTTTACTAACTCTTAAT 3238
F.n.o. LADL--07-285A       ..............T............................................. 3238
F. philomiragia ATCC 25017 ...............................T............................ 3238
F. novicida U112           .G.C..........GCGA......C....A.TT.....C.....GC..T........... 3226
F.t. holarctica LVS        .G............GCGA......C....A.TT.....C..C..GC..T........... 3226
F.t.t. Schu S4             .G............GCGA......C....A.TT.....C..C..GC..T........... 3226
Consensus                  ANGTTAGATAGAGGGNNNTTAGTANCAGANATNTATGANTTTCCNNTANTAACTCTTAAT

                                              3 260                         3 280                         3 300
F.n.n. FSC769              CACTATTTGATGCATGATACTTTTATAAAGCTCAATAGAATGGTTTCTGAATTAAAATCT 3298
F.n.o. LADL--07-285A       .......C...........T.....................A.................. 3298
F. philomiragia ATCC 25017 ............T............................................... 3298
F. novicida U112           ..T....ATT.AGG.....T....G....A..T.....G.CT.........C....G... 3286
F.t. holarctica LVS        ..T....ATT.AGG.....T....G....A..T.....G.CT.........C....G... 3286
F.t.t. Schu S4             ..T....ATT.AGG.....T....G....A..T.....G.CT.........C....G... 3286
Consensus                  CANTATTANNTNGNTGATATTTTTNTAAANCTNAATAGNANTGTTTCTGAANTAAANTCT

                                              3 320                         3 340                         3 360
F.n.n. FSC769              TTCAATCGTTTTGTTTTCTCAACCTCAAGATCATATGCTGCAATTTTACTTGTATTCTTA 3358
F.n.o. LADL--07-285A       ............................................................ 3358
F. philomiragia ATCC 25017 ............................................................ 3358
F. novicida U112           ..T.....C........T...G.T........T.....GT....A...........T..G 3346
F.t. holarctica LVS        ..T.....C........T...G.T........T.....GT....A...........T..G 3346
F.t.t. Schu S4             ..T.....C........T...G.T........T.....GT....A...........T..G 3346
Consensus                  TTNAATCGNTTTGTTTTNTCANCNTCAAGATCNTATGCNNCAATNTTACTTGTATTNTTN
```

```
                                                 3 380                         3 400                        3 420
F.n.n. FSC769               ATTAATAAATTAGAAAGAGAACTTAAATTTGCTGAATCTAATAAGTTAAATAGTTCTCCT 3418
F.n.o. LADL--07-285A        ...................................................G........ 3418
F. philomiragia ATCC 25017  ............................................................ 3418
F. novicida U112            .....................T.G..G.....G..........G.GC.........C..G 3406
F.t. holarctica LVS         .....................T.G..G..C..A..........G.GC.........C..G 3406
F.t.t. Schu S4              .....................T.G..G..C..A..........G.GC........AC..G 3406
Consensus                   ATTAATAAATTAGAAAGAGAANTNAANTTTGCTGAATCTAATANGNNAAATAGTTCNCCN

                                                 3 440                         3 460                        3 480
F.n.n. FSC769               AAGCAAATATTTGATCTAATTCATGATATTTATAGTTTAATCCAGCTTAACTTAGATAAG 3478
F.n.o. LADL--07-285A        ..A...............G......................................... 3478
F. philomiragia ATCC 25017  ............................................A............... 3478
F. novicida U112            ..A............T.....G..........C...C....T..A......C.......A 3466
F.t. holarctica LVS         ..A............T.....G..........C...C....T..A......C.......A 3466
F.t.t. Schu S4              ..A............T.....G..........C...C....T..A......C.......A 3466
Consensus                   AAACAAATATTTGATNTAATTNATGATATTTANAGNTTAATNCAACTTAACNTAGATAAN

                                                 3 500                         3 520                        3 540
F.n.n. FSC769               GTTGGGGATGTTGATAATATTGAGTTTGATTTTTATAAATCTATAAGGAAGATAAACTTA 3538
F.n.o. LADL--07-285A        ....A..................................C.................... 3538
F. philomiragia ATCC 25017  ....A...........G.............................TA............ 3538
F. novicida U112            ....AA..GC......GC.....A........CC.A..GC..T.G.CT..AT....TC.. 3526
F.t. holarctica LVS         ....AA..GC......GC.....A........CC.A..GC..T.G.CT..AT....TC.. 3526
F.t.t. Schu S4              ....AA..GC......GC.....A........CC.A..GC..T.G.CT..AT....TC.. 3526
Consensus                   GTTGANGANNTTGATAGNATTGANTTTGATTTNNANAANCCTNTNACTAANNTAAANNTA

                                                 3 560                         3 580                        3 600
F.n.n. FSC769               TTGGCTGATAGATTATTGACTCTTTGTGAATATAGAAAAATTAATAACTTTATTAAATTT 3598
F.n.o. LADL--07-285A        ......C..................................................... 3598
F. philomiragia ATCC 25017  ............................................................ 3598
F. novicida U112            C.T...........G..A...........G........G..............C.G.... 3586
F.t. holarctica LVS         C.T..............A...........G........G..............C.G.... 3586
F.t.t. Schu S4              C.T...........G..A...........G........G..............C.G.... 3586
Consensus                   NTNGCTGATAGATTATTNACTCTTTGTGANTATAGAAANATTAATAACTTTATNANATTT

                                                 3 620                         3 640                        3 660
F.n.n. FSC769               GAACTGCAAGGAAAAAAATATTTATGTGAAAACTTCCCCGAAGAGTTTTTCGTTGCTACT 3658
F.n.o. LADL--07-285A        ............................................A............... 3658
F. philomiragia ATCC 25017  ............................................................ 3658
F. novicida U112            ...T....T............A.........G...T..T...........T......... 3646
F.t. holarctica LVS         ...T....T............A.........G...T..T...........T......... 3646
F.t.t. Schu S4              ...T....T............A.........G...T..T...........T......... 3646
Consensus                   GAANTGCANGGAAAAAAATATNTATGTGAAANCTTNCCNGAAGAGTTTTTNGTTGCTACT

                                                 3 680                         3 700                        3 720
F.n.n. FSC769               AGGTATTATCTTTTCATCAAAAGAAAGGCAATAGCTCCAGCTAATGTAAAATTTGAAAAT 3718
F.n.o. LADL--07-285A        ..A......A.......T.............C..T......................... 3718
F. philomiragia ATCC 25017  .........A.................................................. 3718
F. novicida U112            ..A..C.........C.T.....G..A....C.........C.......GG......... 3706
F.t. holarctica LVS         ..A..C.........C.T.....G..A....C.........C.......GG......... 3706
F.t.t. Schu S4              ..A..C.........C.T.....G..A....C.........C.......GG......... 3706
Consensus                   AGATANTATCTTTTCNTTAAAAGNAANGCAACAGCTCCAGCNAATGTAANNTTTGAAAAT

                                                 3 740                         3 760                        3 780
F.n.n. FSC769               AAAAAAGCTATGAGAATAACAAGTATAAGTAGAAATAAAAACGTTGTAACTCTCTCTCTT 3778
F.n.o. LADL--07-285A        ............................................................ 3778
F. philomiragia ATCC 25017  ............................................................ 3778
F. novicida U112            ..G..T...C.......T..T.................G..TA........T.G...... 3766
F.t. holarctica LVS         ..G..T...C.......T..T.................G..TA.....G....G...... 3766
F.t.t. Schu S4              ..G..T...C.......T..T.................G..TA.....G....G...... 3766
Consensus                   AANAANGCTNTGAGAATNACNAGTATAAGTAGAAATAANAANNTTGTAACTCTNTCTCTT

                                                 3 800                         3 820                        3 840
F.n.n. FSC769               TCTGGAGTGAAACTCGTTGAAGTTGAGTATTCTATGATGAATTTTACAACACGGGTTGAT 3838
F.n.o. LADL--07-285A        ...........................C.C.............................. 3838
F. philomiragia ATCC 25017  ........T.....T............C.C.............................. 3838
F. novicida U112            ..A.....A.....G.....C.....A.G.........T...........TA.AT..... 3826
F.t. holarctica LVS         ..A.....A.....G.....C.....A.G.........T...........TA.AT..... 3826
F.t.t. Schu S4              ..A.....A.....G.....C.....A.G.........T...........TA.AT..... 3826
Consensus                   TCNGGAGTAAAACTGGTTGANGTTGANTNTTCTATGATNAATTTTACAACNNGNNTTGAT
```

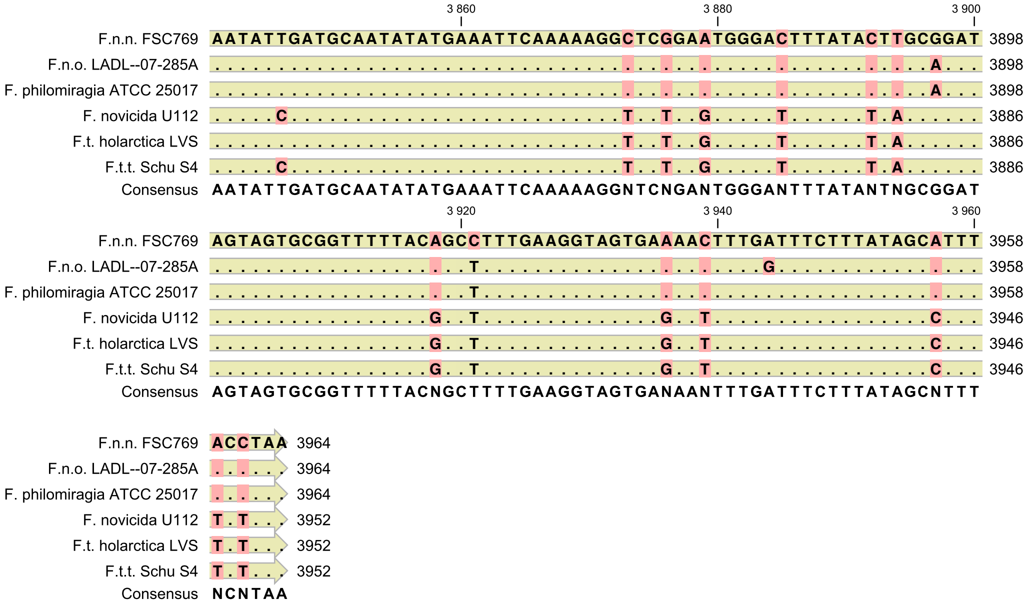
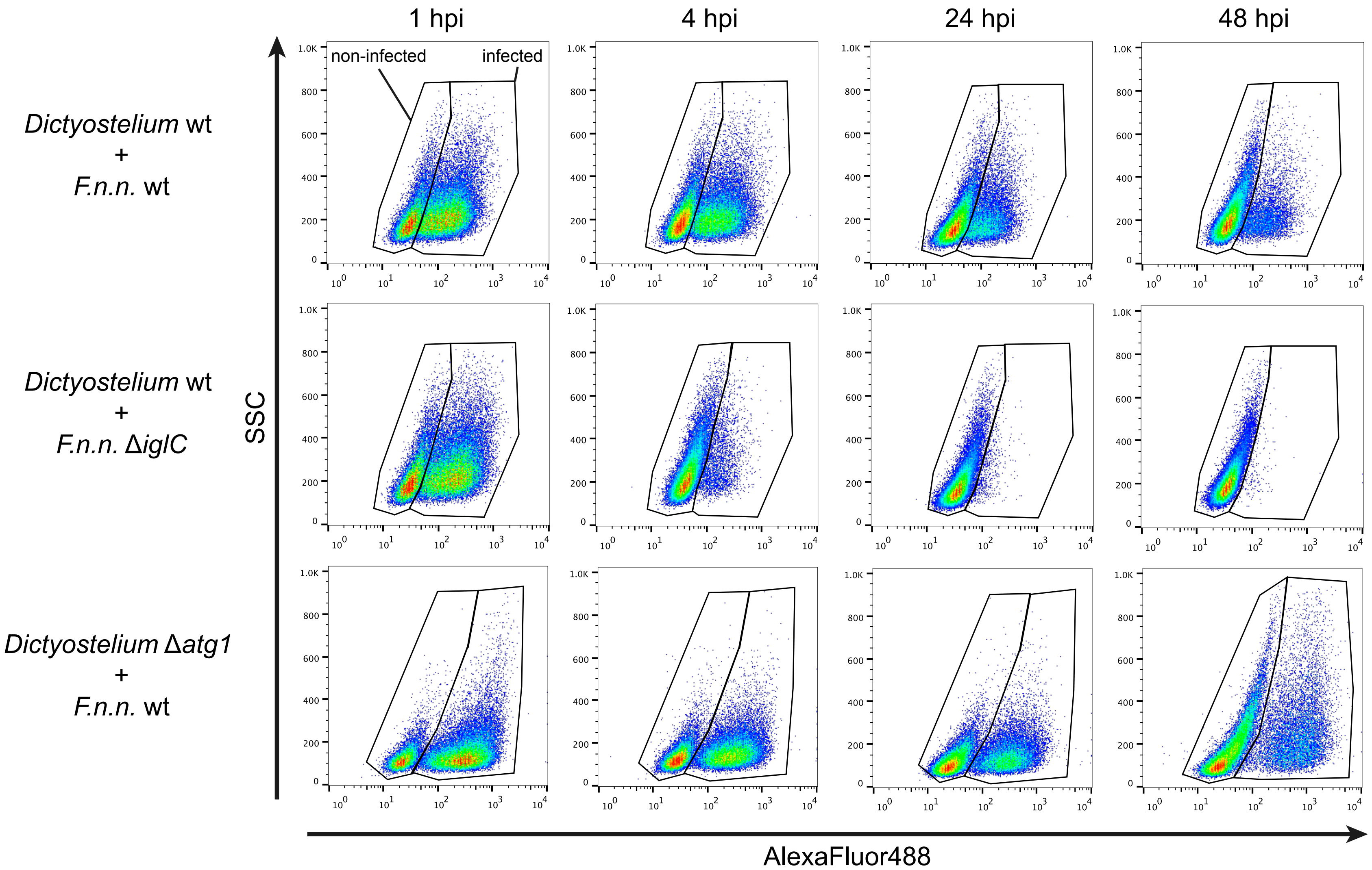
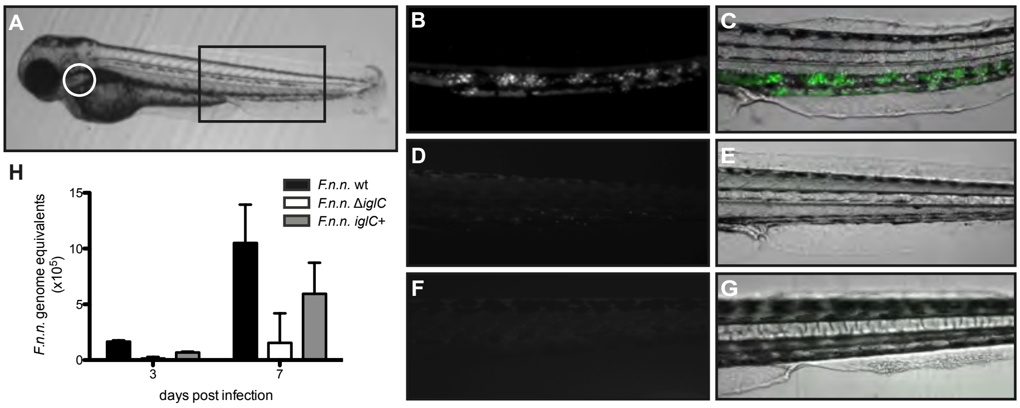

3 860
3 880
3 900
F.n.n. FSC769 AATATTGATGCAATATATGAAATTCAAAAAGGCTCGGAATGGGACTTTATACTTGCGGAT 3898
F.n.o. LADL--07-285A ........................................................A... 3898
F. philomiragia ATCC 25017 ........................................................A... 3898
F. novicida U112 .....C..........................T..T..G.....T......T.A...... 3886
F.t. holarctica LVS ................................T..T..G.....T......T.A...... 3886
F.t.t. Schu S4 .....C..........................T..T..G.....T......T.A...... 3886
Consensus AATATTGATGCAATATATGAAATTCAAAAAGGNTCNGANTGGGANTTTATANTNGCGGAT
3 920
3 940
3 960
F.n.n. FSC769 AGTAGTGCGGTTTTTACAGCCTTTGAAGGTAGTGAAAACTTTGATTTCTTTATAGCATTT 3958
F.n.o. LADL--07-285A ....................T......................G................ 3958
F. philomiragia ATCC 25017 ....................T....................................... 3958
F. novicida U112 .................G..T..............G..T.................C... 3946
F.t. holarctica LVS .................G..T..............G..T.................C... 3946
F.t.t. Schu S4 .................G..T..............G..T.................C... 3946
Consensus AGTAGTGCGGTTTTTACNGCTTTTGAAGGTAGTGANAANTTTGATTTCTTTATAGCNTTT
F.n.n. FSC769 ACCTAA 3964
F.n.o. LADL--07-285A ...... 3964
F. philomiragia ATCC 25017 ...... 3964
F. novicida U112 T.T... 3952
F.t. holarctica LVS T.T... 3952
F.t.t. Schu S4 T.T... 3952
Consensus NCNTAA

**FIG S2**


1 hpi
4 hpi
24 hpi
48 hpi
non-infected
infected
*Dictyostelium* wt + *F.n.n.* wt
*Dictyostelium* wt + *F.n.n.* Δ*iglC*
*Dictyostelium* Δ*atg1* + *F.n.n.* wt
SSC
AlexaFluor488

# FIG S3



A
B
C
D
E
F
G
H
F.n.n. wt
F.n.n. ΔiglC
F.n.n. iglC+
F.n.n. genome equivalents (x10^5)
15
10
5
0
3
7
days post infection

**FIG S4**

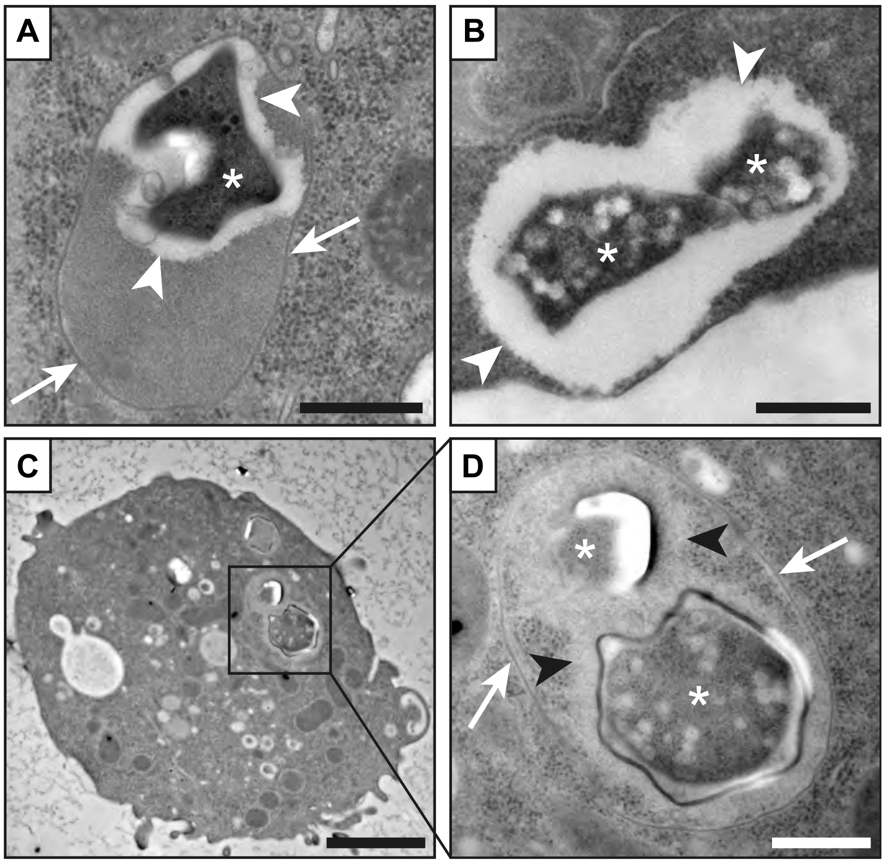


**FIG S5**

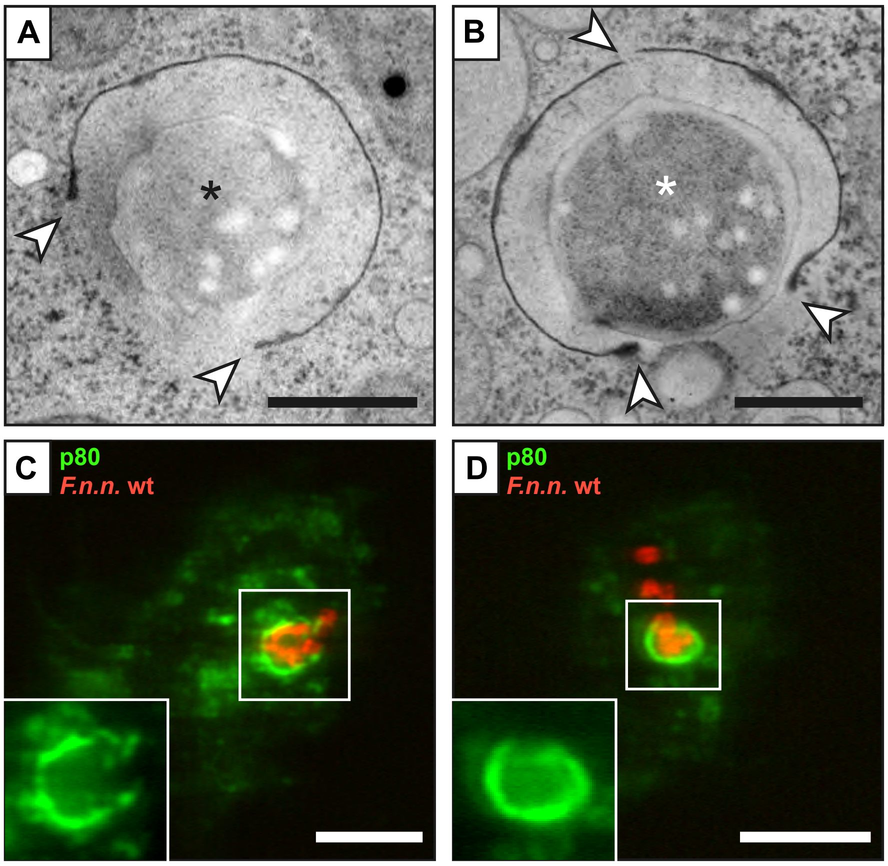
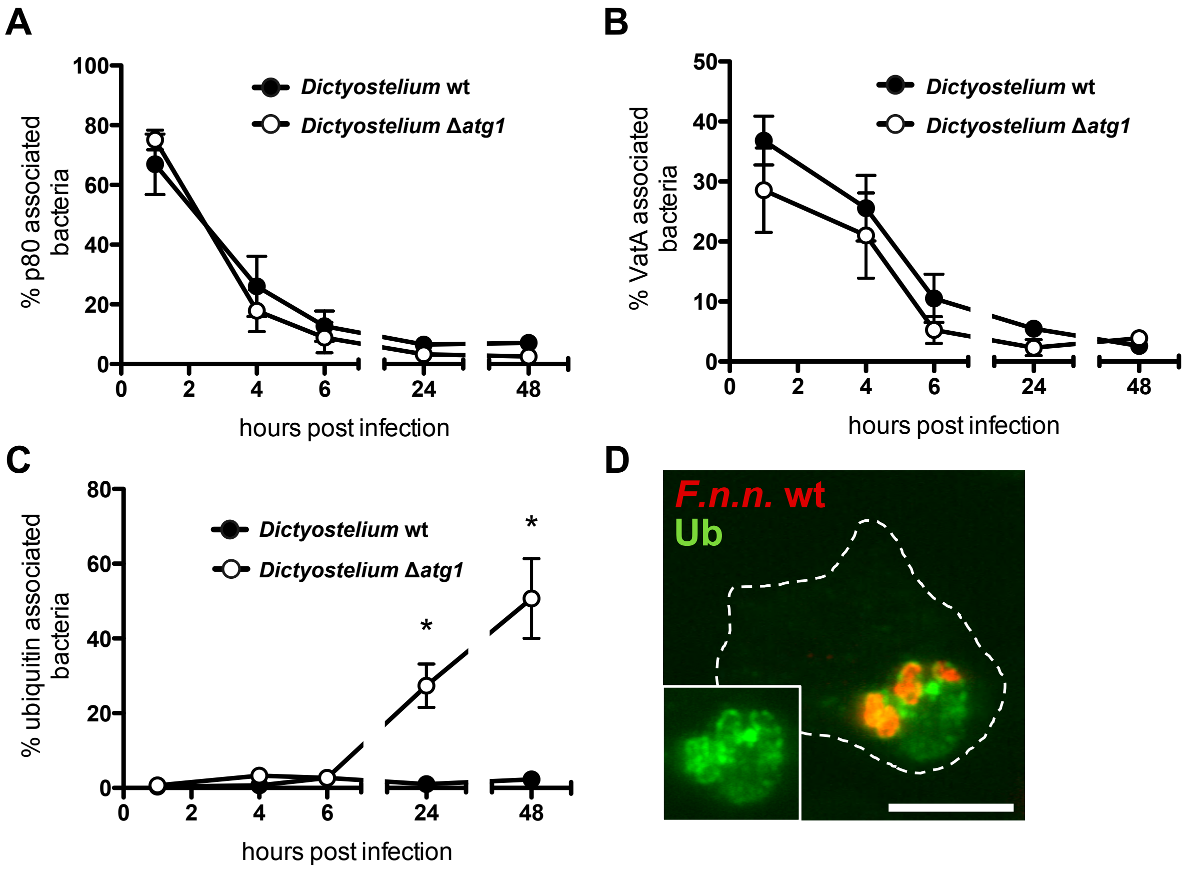


FIG S6


A
% p80 associated bacteria
Dictyostelium wt
Dictyostelium Δatg1
hours post infection
B
% VatA associated bacteria
Dictyostelium wt
Dictyostelium Δatg1
hours post infection
C
% ubiquitin associated bacteria
Dictyostelium wt
Dictyostelium Δatg1
hours post infection
D
F.n.n. wt
Ub

| Primers used for cloning | | | | |
|---|---|---|---|---|
| | **Name** | **5'-3' sequence** | **Product size** | **Source** |
| **P1** | Fnn_iglC_OF | GGTGGTGAACCATATGGCGC | P1+2: 2271 bp in mutant, 2837 bp in wt *F.n.n.* | This study |
| **P2** | Fnn_iglC_OR | TAAAGTCCCATTCCGAGCCTT | | |
| **P3** | Fnn_iglC_IF | CACAGCAAGTTGCTAGAATAGGGTACATAGCAGCT | P1+4: 1184 bp | This study |
| **P4** | Fnn_iglC_IR | GCTATTCTAGCAACTTGCTGTCTTGTTATCATCTC | P2+3: 1105 bp | This study |
| **P5** | Fnn_iglC_C1F | GTGTATGTTGCAGAATTTGATCA | P2+5: 2298 bp in mutant, 2864 bp in wt *F.n.n.* | This study |
| **P6** | Fnn_iglC_C2R | AACCGCACTACTATCCGCAAG | P1+6: 2294 bp in mutant, 2860 bp in wt *F.n.n.* | This study |
| **P7** | Fnn_iglD_IR | TC AAA GGC TGT TCT TAG ACC ATC TTC CCA ACA | P5+7: 1228 bp in mutant, 1794 in wt *F.n.n.* | This study |
| **P8** | Fn_groEL F | CATAAGACCAGCGATTGATGC | 367 bp | This study |
| **P9** | Fn_groEL R | AAAAGATCGTGTGGATGATGC | | |
| **P10** | Fnn_compl_tr_iglC_F(NdeI) | TCT GCA <u>CAT ATG</u> ATT ATG AAC GAG ATG ATA ACA AGA CAG | 635 bp | This study |
| **P11** | Fnn_compl_tr_iglC_R(EcoRI) | CTGA <u>GAA TTC</u> CTA TAC AGC TGC TAT GTA CCC TAT TCT | | |
| **Primers used for qPCR** | | | | |
| | **Name** | **5'-3' sequence** | **Product size** | **Source** |
| **Q1** | Dd_rnlA_F | GGCGCTGGTGAAATAGTAAGTATATTAGA | 85 bp | (1) |
| **Q2** | Dd_rnlA_R | GGTTACCGCCCCAGTCAAA | | |
| **Q3** | Dd_gapdh_F | GGTTGTCCCAATTGGTATTAATGG | 247 bp with gDNA, 156 bp with cDNA | (2) |
| **Q4** | Dd_gapdh_R | CCGTGGGTTGAATCATATTTGAAC | | |
| **Q5** | Dd_atg8_F | CTCCAAGATCAGATGCACCA | 163 bp | Frauke Bach (GI: 8625555) |
| **Q6** | Dd_atg8_R | GCAGCAGTTGGTGGGATAGT | | |
| **Q7** | Dd_p62_F | TTGAAAATCGCACAACCAAC | 172 bp | Frauke Bach (GI: 8617503) |
| **Q8** | Dd_p62_R | AGGAACCCTTTGGAATGACA | | |
| **Q9** | Fnn spec_F | TGAGTTGGTAACCATTGATTGTACATAGT | 97 bp | (3) |
| **Q10** | Fnn spec_R | CGAGTACCTGGTGGGAGAAAGA | | |
| **Q11** | Fnn_iglB_F | GGTGCTGAGCAAATTCAAAA | 85 bp | This study (GI:169589433) |
| **Q12** | Fnn_iglB_R | CCATCTCTAAAGGCGTTGGT | | |
| **Q13** | Fnn_iglC_F | TAGGCGTATAACACTGGCTGC | 70 bp | (4) |
| **Q14** | Fnn_iglC_R | TGCTATAGAAGGCGGAGAGG | | |
| **Q15** | Fnn_iglD_F | TGTGAAAACTTCCCCGAAGA | 77 bp | This study (GI:169589431) |
| **Q16** | Fnn_iglD_R | GCTGGAGCTATTGCCTTTCT | | |

**References**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3. **Duodu S, Larsson P, Sjodin A, Soto E, Forsman M, Colquhoun DJ.** 2012. Real-time PCR assays targeting unique DNA sequences of fish-pathogenic Francisella noatunensis subspecies noatunensis and orientalis. Dis Aquat Organ **101:**225-234.
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