The following supplement accompanies the article

Francisella noatunensis ssp. *noatunensis iglC* deletion mutant protects adult zebrafish challenged with acute mortality dose of wild-type strain

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Supplementary materials and methods

Several published RT-qPCR primers for immune response related carp genes (*ifn*, *socs*: Wang et al. 2013; *tnfα*: Xiao et al. 2010; *il1β*: MacKenzie et al. 2009; *il6*, *il10*: Ouyang et al. 2013; *inos*: Chadzinska et al. 2008) were tested for their transcription in CLC cells and in CLC cells infected with *Francisella noatunensis ssp. noatunensis* (*Fnn*) as these genes have been studied previously in other animal species infected with *Francisella* sp. Unfortunately, transcription was only detected for *tnfα* and the reference genes *40SrRNA* (Chadzinska et al. 2008) and *18SrRNA* (Wang et al. 2013).

Supplementary figures and tables

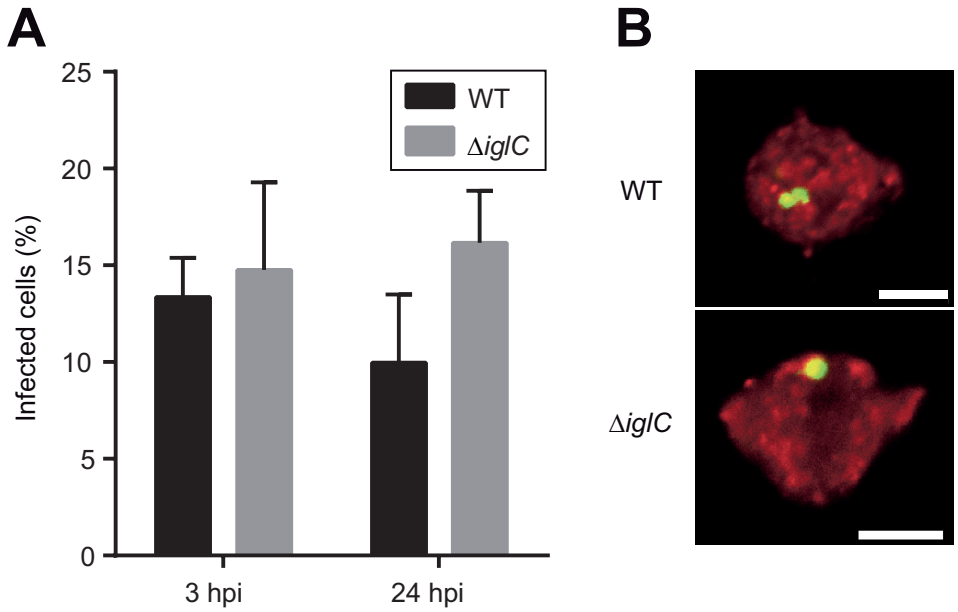

Fig. S1. *Fnn* WT and *Fnn* Δ*iglC* mutant infection in CLC cells. (A) Quantification of *Fnn* WT or *Fnn* Δ*iglC* as percentage of infected CLC cells measured by manual counting on immunolabeled samples by fluorescence microscope. Results are presented as mean ± SEM. (B) Micrographs of CLC cells labeled with Alexa Fluor® 594 conjugated Wheat Germ Agglutinin (red) infected with *Fnn* WT (pKK289Km::*gfp*) or *Fnn* Δ*iglC* (pKK289Km::*gfp*) labeled with α-gfp and Alexa Fluor® 488 conjugated secondary antibodies (green). Scale bars = 5 µm

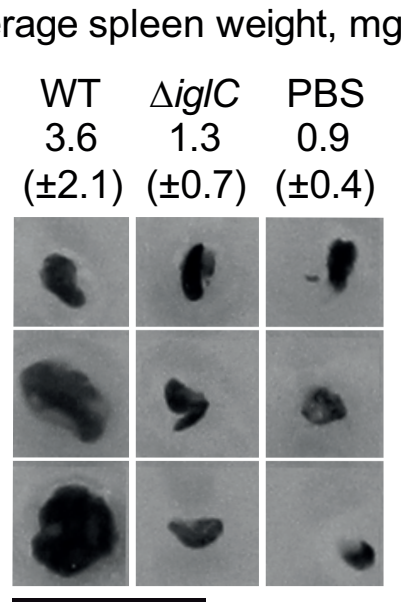

Fig. S2. Splenomegaly of adult zebrafish infected with lower dose *Fnn* WT versus Δ*iglC* infected or PBS injected fish 14 dpi. Six fish per group were sampled from which three representative spleens from each group are presented. Scale bar = 1 cm

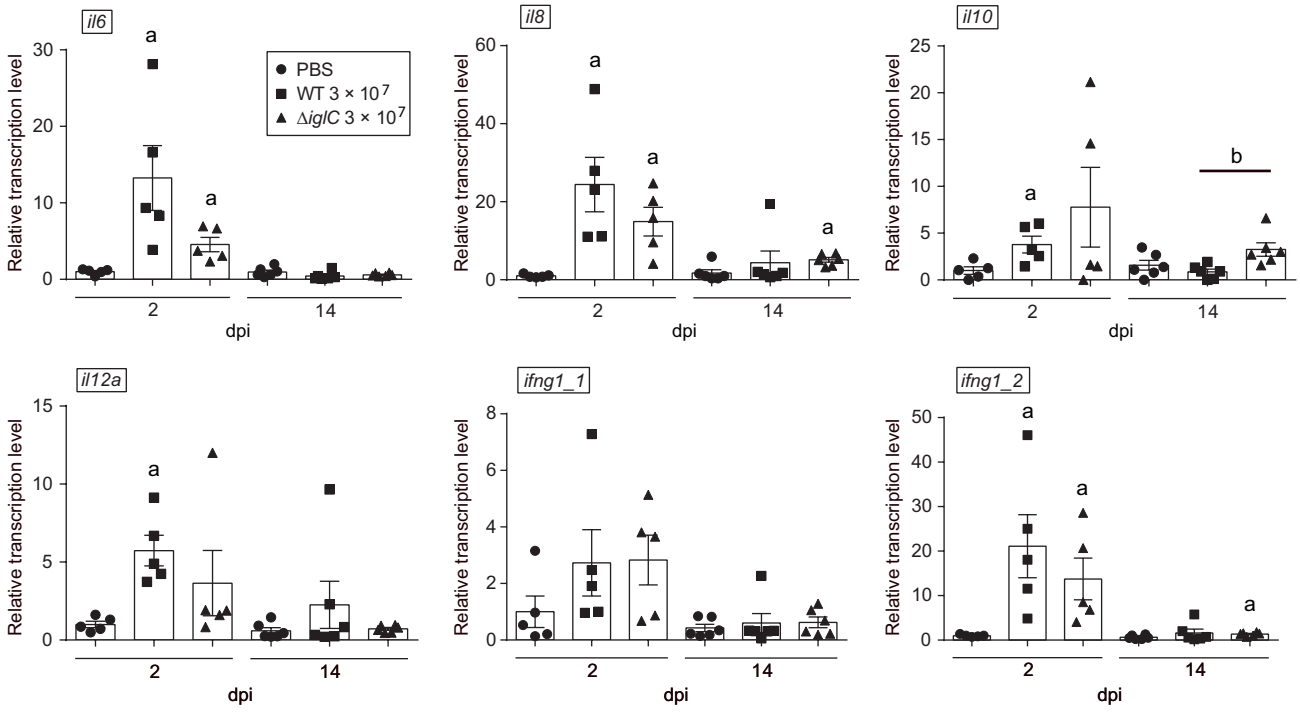

Fig. S3. Immune responses in adult zebrafish during lower dose (3×10^7 CFU) experiment. RT-qPCR was performed on RNA extracted from kidneys of each group at 2 dpi (n = 5) and 14 dpi (n = 6). Results are presented as mean ± SEM with significance level $p < 0.05$. [a]Significant difference from PBS control at timepoint. [b]Significant difference between infected groups at timepoint

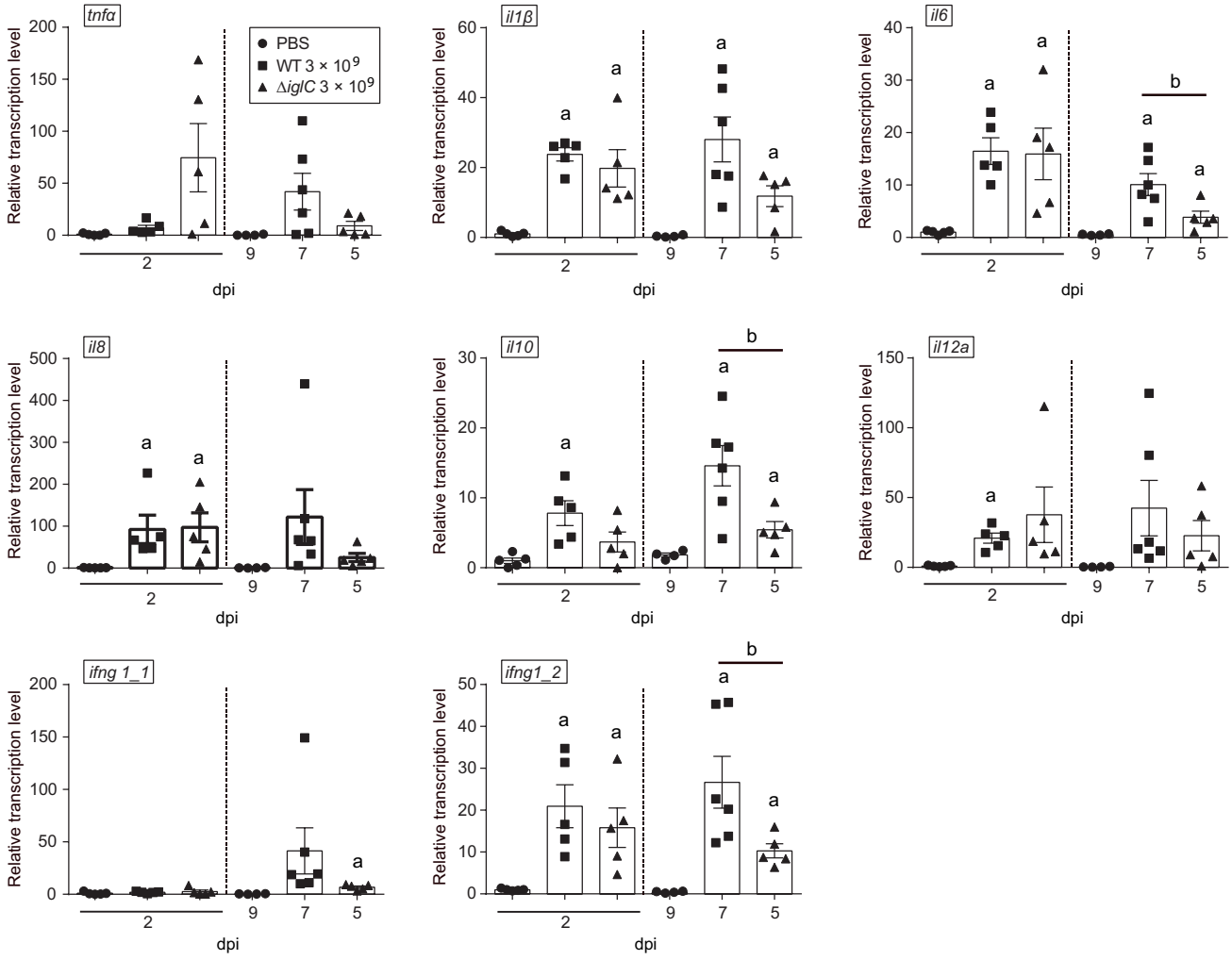

Fig. S4. Immune responses in adult zebrafish during high dose (3×10^9 CFU) experiment. RT-qPCR was performed on RNA extracted from kidneys of each group at 2 dpi (n = 5), 9 dpi for PBS injected fish (n = 4) and at the endpoint of infected groups. The endpoint was 7 dpi (n = 6) and 5 dpi (n = 5) for the *Fnn* WT and *Fnn* Δ*iglC*, respectively. Results are presented as mean ± SEM with significance level $p < 0.05$. [a]Significantly different from PBS control at closest timepoint. [b]Significant difference between infected groups at closest timepoint. The vertical dotted line separates the common early sampling time at 2 dpi from the different endpoints. Significance calculated between the different endpoints are interpreted with caution

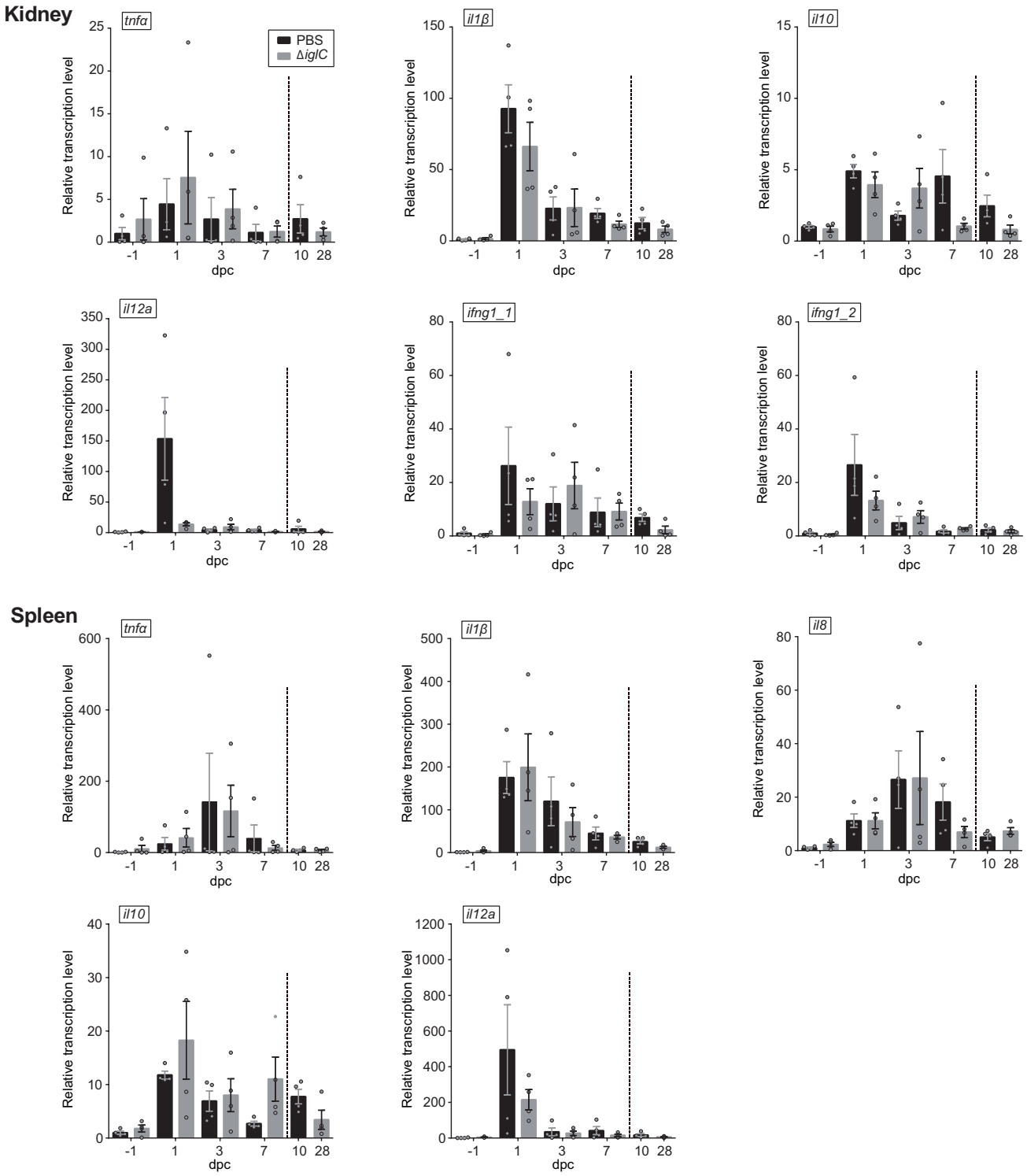

Fig. S5. Immune responses in adult zebrafish after immunization with *Fnn* Δ*iglC* and challenge with *Fnn* WT. RT-qPCR was performed on RNA extracted from spleen and kidneys from four sampled fish per group 1 dbc, 1, 3, 7 dpc and at the endpoint of both groups, which was 10 dpc for non-immunized fish and 28 dpc for mutant immunized fish. Results are presented as mean ± SEM. The vertical dotted line separates the early sampling days from the different endpoints

Table S1. Primers used for qPCR in this study

	Name	Sequence (5'–3')	Product size (bp)	Reference
Q1	Fnn spes_fw	TGAGTTGGTAACCATTGATTGTACATAGT	97	Duodu et al. (2012)
Q2	Fnn spes_rev	CGAGTACCTGGTGGGAGAAAGA		
Q9	ef1α (cod)_F	ATGTGAGCGGTGTGGCAATC	72	Seppola et al. (2008)
Q10	ef1α (cod)_R	TCATCATCCTGAACCACCCTG		
Q11	il1β (cod)_F	GGAGAACACGGACGACCTGA	50	Seppola et al. (2008)
Q12	il1β (cod)_R	CGCACCATGTCACTGTCCTT		
Q13	il6 (cod)_F	TGAAGAAGGAGTACCCCGACAAT	92	Bakkemo et al. (2012)
Q14	il6 (cod)_R	GGTGCCTCATCTTTTCCTCAATG		
Q15	il8 (cod)_F	GGTTTGTTCAATGATGGGCTGTT	70	Seppola et al. (2008)
Q16	il8 (cod)_R	GACCTTGCCTCCTCATGGTAATACT		
Q17	il10 (cod)_F	CCTATAAAGCCATCGGCGAGTTA	75	Seppola et al. (2008)
Q18	il10 (cod)_R	TGAAGTCGTCGTTTTGAACCAAG		
Q19	ef1a (zf)_F	CTTCTCAGGCTGACTGTGC	358	McCurley & Callard (2008)
Q20	ef1a (zf)_R	CCGCTAGCATTACCCTCC		
Q21	18S (zf)_F	ACCACCCACAGAATCGAGAAA	98	Dios et al. (2010)
Q22	18S (zf)_R	GCCTGCGGCTTAATTTGACT		
Q23	β-actin (zf)	QuantiTect Primer Assay Dr_actb1_1_SG	142	Qiagen, cat# QT02174907
Q24	il1β (zf)	QuantiTect Primer Assay Dr_il1b_1_SG	111	Qiagen, cat# QT02063565
Q25	tnfa (zf)	QuantiTect Primer Assay Dr_tnfa_1_SG	81	Qiagen, cat# QT02097655
Q26	il6 (zf)_F	TCAACTTCTCCAGCGTGATG	73	Varela et al. (2012)
Q27	il6 (zf)_R	TCTTTCCCTCTTTTCCTCCTG		
Q28	il8 (zf)	QuantiTect Primer Assay Dr_il8_1_SG	147	Qiagen, cat# QT02108190
Q29	il10 (zf)	QuantiTect Primer Assay Dr_il10_1_SG	144	Qiagen, cat# QT02063922
Q30	il12a (zf)	QuantiTect Primer Assay Dr_il12a_1_SG	94	Qiagen, cat# QT02085300
Q31	ifng1_1 (zf)	QuantiTect Primer Assay Dr_ifng1-1_1_SG	116	Qiagen, cat# QT02151961
Q32	ifng1_2 (zf)	QuantiTect Primer Assay Dr_ifng1-2_1_SG	89	Qiagen, cat# QT02064328
Q33	il1b (zf_gDNA)_F	TTCCCCAAGTGCTGCTTATT	149	Dios et al. (2010)
Q34	il1b (zf_gDNA)_R	AAGTTAAAACCGCTGTGGTCA		

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