

## Supplemental Material

### **Incompatibility of *Vibrio fischeri* strains during symbiosis establishment depends on two functionally redundant *hcp* genes**

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VFMJ11_1495      MPTPAYMSIKGETQGDITKDAYSAHSVGNVQEAHVDEFLVQELDHVLTVPRDPQSGQPT 60
VFFQA001_Hcp    MPTPAYMSIKGETQGDITKDAYSAHSVGNVQEAHVDEFLVQELDHVLTVPRDPQSGQPT 60
VFFQA001_Hcp1   MPTPAYMSIKGETQGDITKDAYSAHSVGNVQEAHVDEFLVQELDHVLTVPRDPQSGQPT 60
VFMJ11_A0831    MPTPAYMSIKGETQGDITKDAYSAHSVGNVQEAHVDEFLVQELDHVLTVPRDPQSGQPT 60
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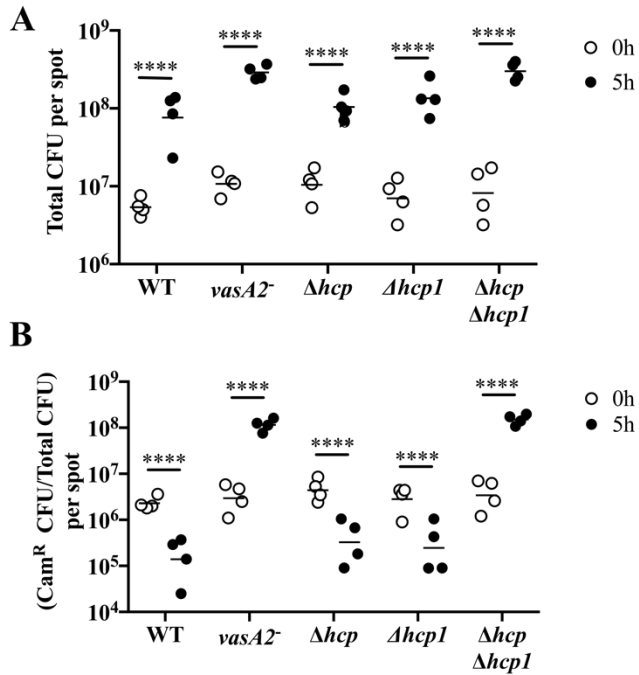
VFMJ11_1495      GQRVHRPVVVTKQQDRCSPLLFNALVSGEKLPECSINFYRTSTSGKQEHYYTIKLIDALL 120
VFFQA001_Hcp    GQRVHRPVVVTKQQDRCSPLLFNALVSGEKLPECSINFYRTSTSGKQEHYYTIKLIDALL 120
VFFQA001_Hcp1   GQRVHRPVVVTKQQDRCSPLLFNALVSGEKLPECSINFYRTSTSGKQEHYYTIKLIDALL 120
VFMJ11_A0831    GQRVHRPVVVTKQQDRCSPLLFNSLVSGEKLPECNIKFYRTSTSGKQEHYYTIKLIDALL 120
*****:*****.*:*****

VFMJ11_1495      VDMQTRMAHCQDAAMADRVTEEVLKFTYRAIEVTHETCGTAGNDDWRTPREA 172
VFFQA001_Hcp    VDMQTRMAHCQDAAMADRVTEEVLKFTYRAIEVTHETCGTAGNDDWRTPREA 172
VFFQA001_Hcp1   VDMQTRMAHCQDAAMADRVTEEVLKFTYRAIEVTHETCGTAGNDDWRTPREA 172
VFMJ11_A0831    VDMQTRMAHCQDAAMSDRVTEEVLKFTYRAIEVTHETCGTAGNDDWRTPREA 172
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**Figure S1. *V. fischeri* Hcp amino acid sequence alignment.**

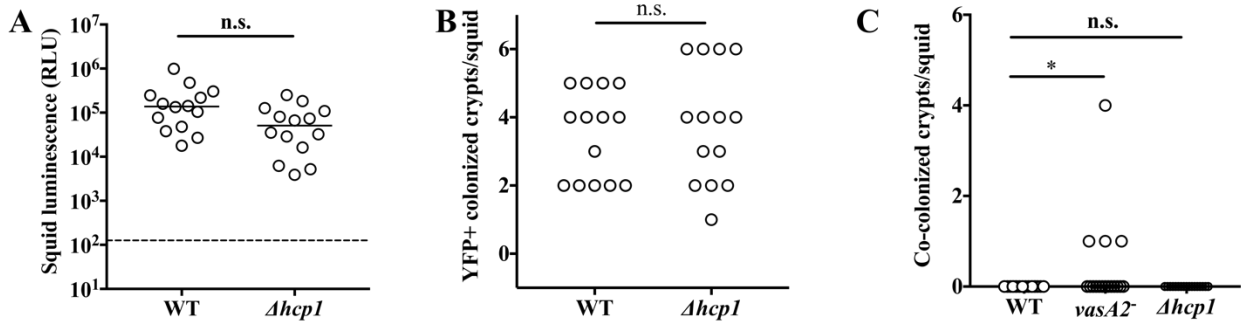
Amino acid sequence alignment of Hcp homologs in FQ-A001 and MJ11. Alignments were generated using Clustal Omega. “\*” indicates conserved residue; “:” indicates residues have strongly similar functional groups; “.” indicates residues have weakly similar functional groups.



**Figure S2. Change in cellular abundance over time for spots described in Fig. 4B.**

A. Total CFU. Two-way ANOVA revealed significant differences among means of log-transformed data over time ( $F_{1,30} = 336.0$ ,  $p < 0.0001$ ), due to genotype ( $F_{4,30} = 5.032$ ,  $p = 0.0032$ ), but not due to their interaction ( $F_{4,30} = 2.022$ ,  $p = 0.1166$ ). A Sidak's *post-hoc* test was performed to statistically compare the log-transformed means between each time point for each group, with  $p$ -values adjusted for multiple comparisons (\*\*\*\* =  $p < 0.0001$ ).

B. Cam<sup>R</sup> CFU. Two-way ANOVA revealed significant differences among means of log-transformed data due to genotype ( $F_{4,30} = 38.11$ ,  $p < 0.0001$ ), not over time ( $F_{1,30} = 0.08613$ ,  $p = 0.7712$ ), but due to their interaction ( $F_{4,30} = 36.36$ ,  $p < 0.0001$ ). A Sidak's *post-hoc* test was performed to statistically compare the log-transformed means between each time point for each group, with  $p$ -values adjusted for multiple comparisons (\*\*\*\* =  $p < 0.0001$ , \*\*\* =  $p < 0.001$ , \*\* =  $p < 0.01$ ).

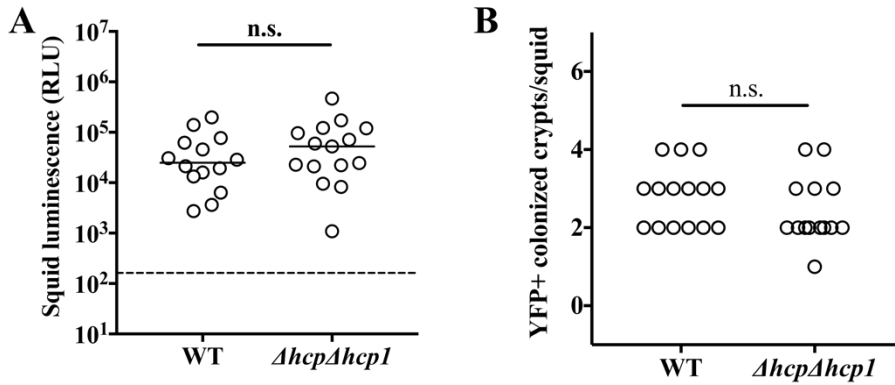


**Figure S3. Deletion of *hcp1* does not impact FQ-A001 symbiosis establishment.**

A. Luminescence of squid 48 h.p.i. 14 animals were used in both groups. Dotted line indicates the threshold for luminescent-positive animals, calculated by performing a one-tailed t test on the luminescence associated with squid that were not exposed to bacteria (apo-symbiotic). No significant differences were observed between groups of squid that were exposed to bacteria (Kruskal-Wallis test  $p \approx 0.0641$ ).

B. Number of crypts colonized per squid. No significant differences were observed between groups (Kruskal-Wallis test  $p \approx 0.82$ ). 14 animals were used in both groups.

C) Number of crypts that were positive for CFP and YFP fluorescence. FQ-A001  $\Delta hcp1$  = NPW57. Between 17-18 animals were used in each group. Kruskal-Wallis test and Dunn's multiple comparison *post-hoc* test was used to determine differences between groups, with \* =  $p < 0.05$ .



**Figure S4. Impact of *hcp* and *hcp1* on symbiosis establishment by FQ-A001.**

A. Luminescence of animals at 48 h after initial exposure to inoculum containing either FQ-A001 (WT) or NPW58 ( $\Delta hcp \Delta hcp1$ ) harboring the YFP-expression plasmid pSCV38. Dotted line indicates the 95% tail of luminescence associated with animals within an apo-symbiotic group, above which animals are scored as luminescent. Between 17-18 animals were used in each group. A Mann-Whitney test determined the medians for luminescent animals within each group are not significantly different between groups ( $\alpha = 0.05$ ). n.s. = not significant ( $p > 0.05$ ).

B. Number of crypts spaces per animal in A exhibiting YFP fluorescence. A Mann-Whitney test determined the medians for animals within each group are not significantly different between groups ( $\alpha = 0.05$ ). n.s. = not significant ( $p > 0.05$ ).