

SUPPLEMENTARY INFORMATION

**A novel flagellar sheath protein, FcpA, determines
filament coiling, translational motility and virulence
for the *Leptospira* spirochete**

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Supplemental tables

Table S1. LD₅₀ of wild-type, *fcpA*⁻ mutant and complemented strains of *L. interrogans* in the hamster model of leptospirosis *

Bacterial Strains	LD ⁵⁰
motile Fiocruz LV2756 ⌘	4.64 leptospores
motility-deficient Fiocruz LV2756 ★	>10 ⁸ leptospores ¶
Fiocruz LV2756 <i>fcpA</i> ^{-/+} §⌘	4.64 leptospores
Fiocruz L1-130 <i>WT</i> ⌘	<10 leptospores ❖
Fiocruz L1-130 <i>fcpA</i> ⁻ ★	>10 ⁸ leptospires ¶
Fiocruz L1-130 <i>fcpA</i> ^{-/+} §⌘	10 leptospores

* Results are shown for one representative experiment of a total of two, which were performed. Groups of 04 animals were inoculated intraperitoneally and followed for 21 days after infection.

¶ All the animals inoculated with 10⁸ leptospores survived.

§ Strains were genetically complemented with *fcpA* gene.

❖ All the animals inoculated with 10 leptospores died.

⌘ Animals were inoculated with 10¹, 10² and 10³ bacteria.

★ Animals were inoculated with 10⁶, 10⁷ and 10⁸ bacteria

Table S2. Sequence of primers used in this study

Primer	Sequence (5'-3')
FcpA_FlkAF	CGGGATCCCGGATTTCTTGGGTCATTTCTT
FcpA_FlkAR	GCTCTAGAGCTTCTCTTTCAATGGTATTAG
FcpA_FlkBF	CCCAAGCTTGGGCGTTCACCTTTTGAGAGCGA
FcpA_FlkBR	GGACTAGTCCGCTTCAATCGACCGTTTCCA
Spc_Xba5	GCTCTAGAAACGCGTCCCGAGC
Spc_Hind3	CCCAAGCTTAACGCGTAAAGTAAGCACC
FcpA_AscF	GGCGCGCCTGGATCATTGAATAGTCTAT
FcpA_AscR	GGCGCGCCAAGGATCTTGGTTCGTAAAA
LipL32-45F	AAGCATTACCGCTTGTGGTG
LiL32-286R	GAACTCCCATTTCAGCGATT
LipL32-189p	[6-FAM]* AAAGCCAGGACAAGCGCCG [BHQ1a-Q]¶
GAPDH_R	GGTTCACACCCATCACAAACAT
GAPDH_F	GGTGGAGCCAAGAGGGTCAT
GAPDH_P	[6-FAM] ATCTCCGCACCTTCTGCTGATGCC [BHQ1a-Q]

* 6-Carboxyfluorescein fluorescent dye

¶ Black Hole Quencher™ 1

Supplemental Figure 1

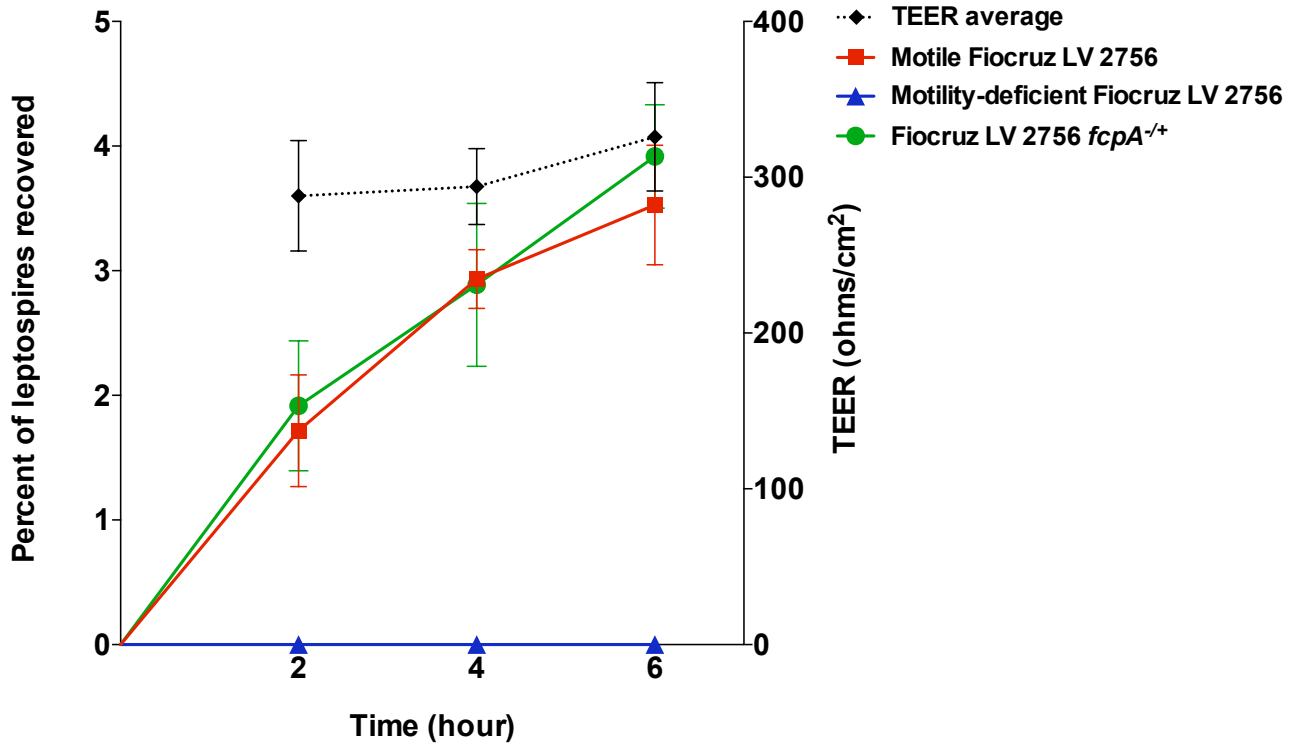


Fig S1. Translocation of motile, motility-deficient and complemented *Leptospira interrogans* strains across polarized MDCK cell monolayers. Motile Fiocruz LV 2756, Motility-deficient LV 2756, and Fiocruz LV 2756 *fcpA*^{+/+} strains were inoculated with a multiplicity of infection of 100 in the upper chamber of a MDCK cell monolayer transwell system. We measured percent recovery of leptospire by counting bacteria in the lower chamber at 2, 4, and 6 hours after inoculation. Results are shown as mean and standard deviation of assay values, which were performed in triplicate. Left Y axis shows TEER measurements.

Supplemental Figure 2

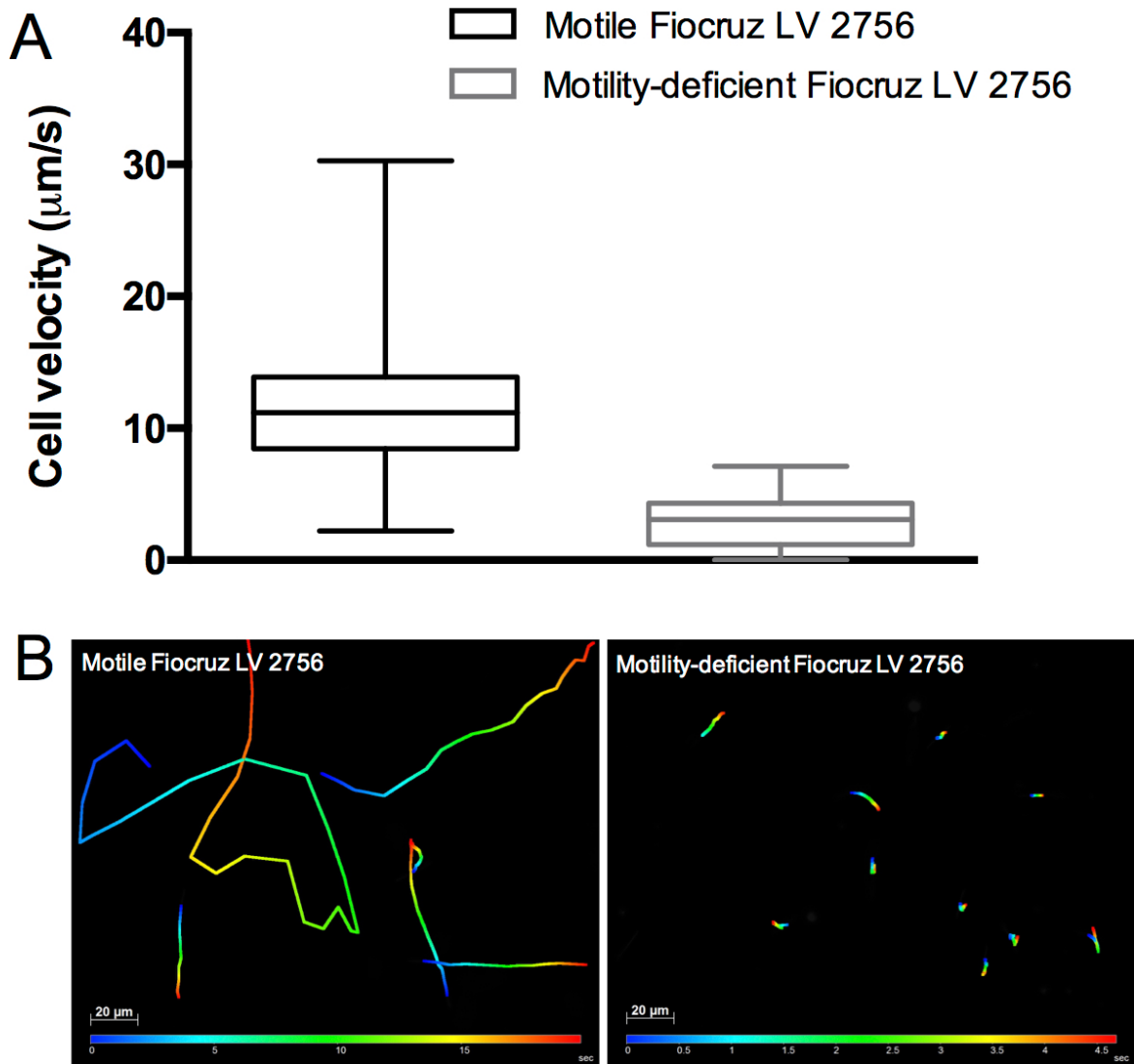


Fig S2. Range of leptospiral cell mean velocities of motile and motility-deficient strains. The mean velocity of Motile Fiocruz LV 2756 and Motility-deficient LV 2756 strains were measured using the tracking module of AxioVision 4.8.2 software (Carl Zeiss). (A) Box-and whisker plot showing the range of leptospiral cell mean velocity. (B) Representative color tracks showing the path of individual leptospires tracked over 10 seconds.

Supplemental Videos

Video S1. Dark-field video microscopy of motile Fiocruz LV2756 strain. Video was generated using a Zeiss AxioImager.M2 microscope outfitted with an AxioCam MRm camera and analyzed images using the AxioVision 4.8.2 software (Carl Zeiss Microscopy LLC). Cells were diluted in 0.5% solution of methylcellulose (MP Biomedicals, LLC) in PBS. The time lapse was recorded and exported at 14 fps.

Video S2. Dark-field video microscopy of motility-deficient Fiocruz LV2756 strain. Video was generated using a Zeiss AxioImager.M2 microscope outfitted with an AxioCam MRm camera and analyzed images using the AxioVision 4.8.2 software (Carl Zeiss Microscopy LLC). Cells were diluted in 0.5% solution of methylcellulose (MP Biomedicals, LLC) in PBS. The time lapse was recorded and exported at 14 fps.

Video S3. Dark-field video microscopy of wild-type Fiocruz L1-130 strain. Video was generated using a Zeiss AxioImager.M2 microscope outfitted with an AxioCam MRm camera and analyzed images using the AxioVision 4.8.2 software (Carl Zeiss Microscopy LLC). Cells were diluted in 0.5% solution of methylcellulose (MP Biomedicals, LLC) in PBS. The time lapse was recorded and exported at 14 fps.

Video S4. Dark-field video microscopy of Fiocruz L1-130 *fcpA*⁻ strain. Video was generated using a Zeiss AxioImager.M2 microscope outfitted with an AxioCam MRm

camera and analyzed images using the AxioVision 4.8.2 software (Carl Zeiss Microscopy LLC). Cells were diluted in 0.5% solution of methylcellulose (MP Biomedicals, LLC) in PBS. The time lapse was recorded and exported at 14 fps.

Video S5. Dark-field video microscopy of complemented Fiocruz LV2756 *fcpA*^{-/+} strain. Video was generated using a Zeiss AxioImager.M2 microscope outfitted with an AxioCam MRm camera and analyzed images using the AxioVision 4.8.2 software (Carl Zeiss Microscopy LLC). Cells were diluted in 0.5% solution of methylcellulose (MP Biomedicals, LLC) in PBS. The time lapse was recorded and exported at 14 fps.

Video S6. Dark-field video microscopy of complemented Fiocruz L1-130 *fcpA*^{-/+} strain. Video was generated using a Zeiss AxioImager.M2 microscope outfitted with an AxioCam MRm camera and analyzed images using the AxioVision 4.8.2 software (Carl Zeiss Microscopy LLC). Cells were diluted in 0.5% solution of methylcellulose (MP Biomedicals, LLC) in PBS. The time lapse was recorded and exported at 14 fps.

Video S7. Three-dimensional reconstruction of mutant Fiocruz L1-130 *fcpA*⁻ strain, shown in Figure 4. Reconstruction of cells were segmented using 3D modeling software Amira (Visage Imaging), as described in Experimental Procedures.

Video S8. Three-dimensional reconstruction of complemented Fiocruz L1-130 *fcpA*^{-/+} strain, shown in Figure 4. Reconstruction of cells were segmented using 3D modeling software Amira (Visage Imaging), as described in Experimental Procedures.