



Supplemental Figure 1

Ear skin tissue transplants from antibiotic-treated mice induce IgG reactivity to select *B.*

burgdorferi antigens in recipient *Myd88*^{-/-} mice. Arrowheads indicate bands of interest. Panel

A) IgG immunoblot of Bb914 lysate using 1:50 dilution of sera from recipient mice 24-28d after tissue transplant. Lane 1, normal mouse serum; lane 2, positive control immune mouse serum from infected B6 *myD88* mouse; lanes 3-6, sera from mice transplanted with sham-treated donor tissue; lanes 7-10, sera from mice transplanted with tissue from doxycycline-treated donors; lanes 11-14, sera from mice transplanted with tissue from uninfected mice. Panel B) IgG immunoblot of Bb914 lysate using sera from the indicated recipient mice. Lanes 1 and 2, negative and positive control sera, respectively; lanes 3 and 4, 1:200 dilution of sera from recipient mice 5 mos after tissue transplant from a doxycycline-treated donor mouse (lane 3) or sham-treated donor (lane 4); lanes 5 and 6, 1:100 dilution of sera from recipient mice 14d after tissue transplant with a ceftriaxone-treated donor mouse (lane 5) or sham-treated donor (lane 6). Panel C) IgG immunoblot of Bb914 lysate using sera from mice immunized with

homogenates of tissue from the same sham-treated mice used as donors in Panel A, lanes 3 and 5. Lanes 1 and 2 are negative and positive control sera, respectively; lanes 3 and 4, 1:100 dilution of sera from mice immunized with homogenates of tissue from sham-treated mice.

Supplemental QuickTime Video Legends

Supplemental Video #1: *GFP⁺Bb914 in the dermis of a sham-treated MyD88^{-/-} mouse at 22d of infection.* A z-stack of images consisting of 16 optical sections of 2 μm thickness was collected every 25 sec over a period of 30 min. Twelve sec of the 19 sec video are shown, corresponding to 19 min of imaging. Second harmonic resonance of the dermal collagen fibers labels them blue. Note the three forms of spirochete movement: directional translocation in which spirochetes move primarily in one direction; oscillation back and forth; and stationary. The density of spirochetes was similar throughout the ear tissue.

Supplemental Video #2: *GFP⁺Bb914 in the calcaneal tendon of a sham-treated mouse at 7 wk of infection.* The tendon has been surgically exposed and the second harmonic generation of the collagen fibers delineates the horizontally organized fibers of the tendon and the less organized fibers of the overlying skin. A single optical section was acquired every sec over a period of 1 min. Note the random orientation of spirochetes in the skin and the more regular orientation of spirochetes along the fibers of the tendon.

Supplemental Video #3: *GFP⁺Bb914 in the dermis of a 22d-infected MyD88^{-/-} mouse 24h after beginning ceftriaxone.* Imaging parameters were identical to those for Supplemental Video #1. Note the dramatic reduction in spirochete numbers in the field. The spirochete in the upper right field stops abruptly and turns into a spherical shape. See Supplemental Video #4 for close-up views in the yz plane.

Supplemental Video #4. The same z-stack acquired as described in Supplemental Video #3, rotated to view the yz plane. The video shows the spirochete changing into a sphere even

when viewed in the yz plane, demonstrating that the change in shape was not due to the long axis of the spirochete shifting from the xy to the yz plane.

Supplemental Video #5. *GFP⁺Bb914 in the dermis of a 22d-infected MyD88^{-/-} mouse 24h after beginning ceftriaxone.* Imaging parameters were identical to Supplemental Video #1 except that z-stack images were acquired over a period of 1h. Fifteen sec of the 36 sec video are shown, corresponding to 25 min of imaging. Note the spirochete in the center flexes once, then rapidly converts to a spherical shape.

Supplemental Video #6: *GFP⁺Bb914 and amorphous GFP⁺ deposits adjacent to ear cartilage in a sham-treated mouse.* A z-stack of images consisting of 61 optical sections of 1 μm thickness was acquired. The cartilage autofluoresces red and the dermis is blue due to second harmonic generation. Several spirochetes are visualized on the surface of the cartilage along with large amorphous GFP⁺ deposits.

Supplemental Video #7: *Z-stack of GFP⁺Bb914 and amorphous GFP⁺ deposits in the quadriceps enthesis of a sham-treated mouse.* A z-stack of images consisting of 101 optical sections of 1 μm thickness was acquired. Intact spirochetes can be seen primarily in the tendon region whereas the amorphous GFP⁺ deposits are closer to the cartilage.