Prashar et al., http://www.jcb.org/cgi/content/full/jcb.201304095/DC1

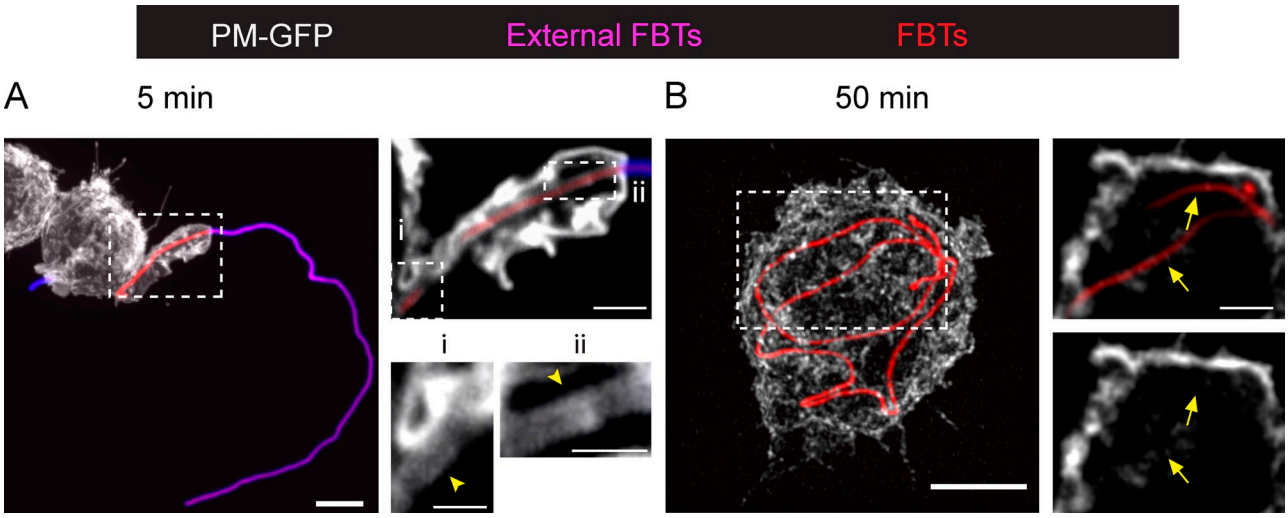


Figure S1. **FBT phagocytosis in cells expressing PM-GFP.** (A) T-PC after 5 min of phagocytosis of FBT in RAW cells expressing PM-GFP. PM-GFP (white) is labeling the T-PCs (arrowheads). Images shown to the right are higher magnification of confocal sections from framed areas. Regions noted as i and ii are enlargements from the indicated panel. (B) Fully internalized FBTs are no longer associated with PM-GFP–positive membranes (arrows) after 50 min of phagocytosis. Bars: (main panels) 5 µm; (magnifications) 2.5 µm.

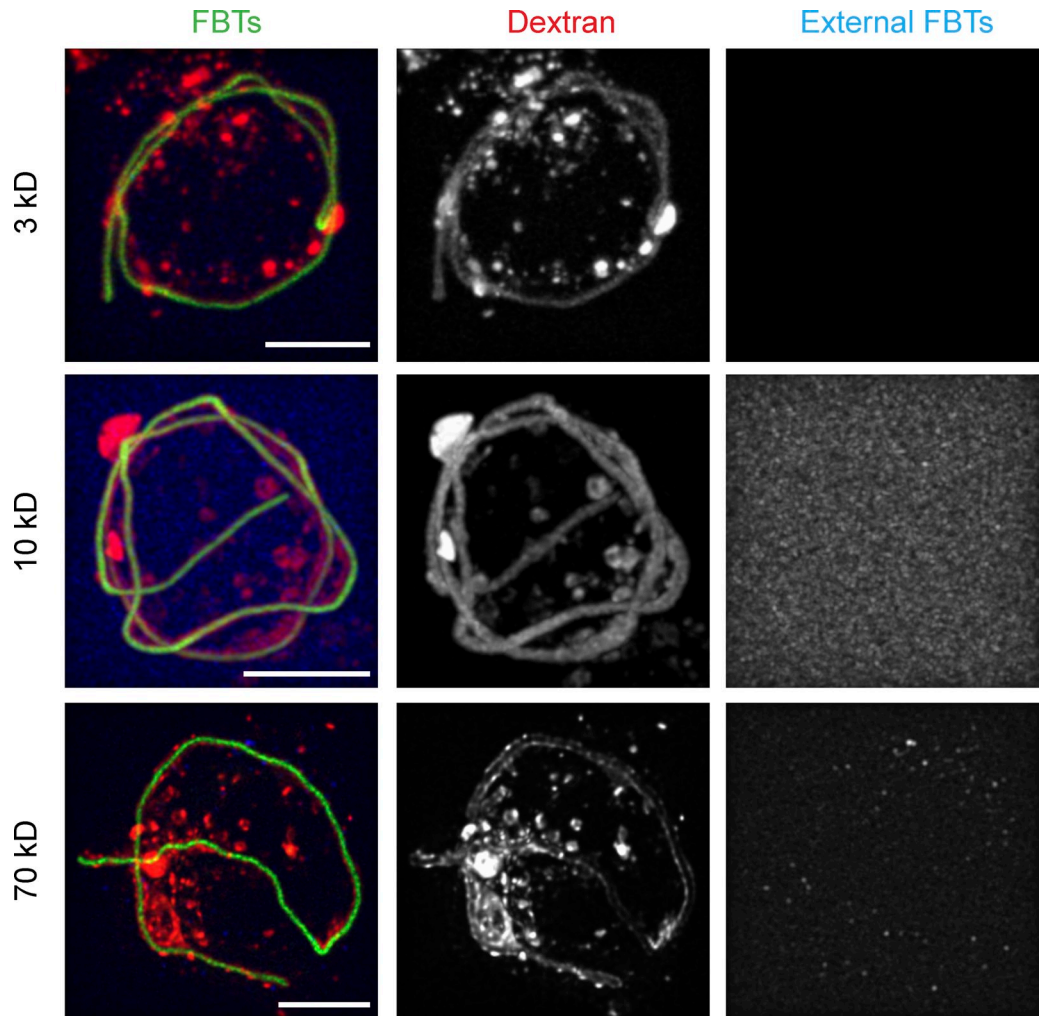


Figure S2. **Fusion of late endosomes and lysosomes with tubular phagosomes.** Images showing fully internalized FBTs in phagosomes that fuse with late endosomes and lysosomes. Endosomal compartments were labeled with fluorescent dextrans of different molecular weights (red) with fluid-phase pulse and chase. Cells were allowed to phagocytose GFP-FBTs by for 20 min, fixed with PFA, and external FBTs were immunolabeled (blue). The molecular weight of the dextrans used is indicated on the left. Bars: (main panels) 5 µm; (magnifications) 2.5 µm.

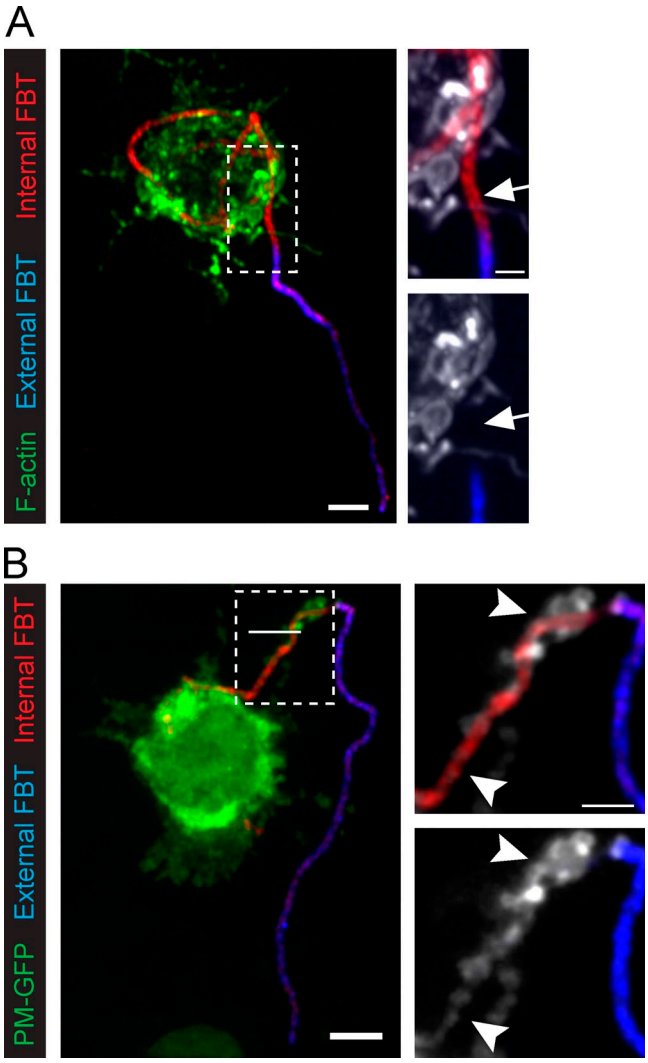


Figure S3. **Phagocytic protuberance persists after actin depolymerization with latrunculin B.** (A) Treatment with latrunculin B disassembled the actin jacket formed after 20 min of phagocytosis. However, the phagocytic protuberance remains in place as per the labeling of the T-PC with PM-GFP (B). Macrophages were presented with unlabeled FBTs and 20 min after the initial attachment external sections of FBTs were labeled (blue). Cells were then treated with latrunculin B (latB) for 20 min and external segments of FBTs labeled again (red). (A) F-actin (green) cannot be detected in a phagocytic protuberance around FBTs as they get internalized. Antibody penetration inside the T-PC after latB treatment is shown in red. Arrows in the magnified images indicate the absence of the actin jacket (white). (B) T-PC labeled with PM-GFP in a cell treated with latB. External FBTs labeled after the first and second round of labeling are shown in blue and red, respectively. Arrowheads in the magnified panels point to the PM-GFP–decorated T-PC (white). Bars: (main panels) 5 µm; (magnifications) 2.5 µm.

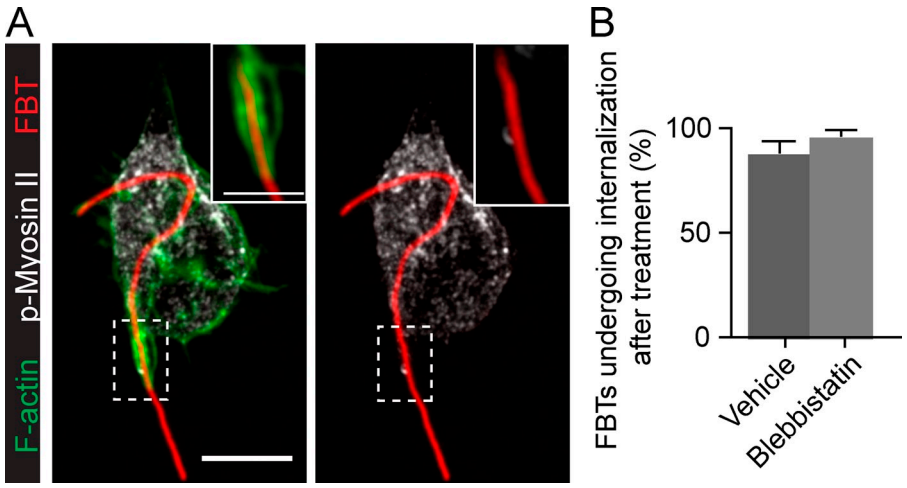


Figure S4. **FBT internalization does not require myosin II.** (A) Phospho-myosin II was not detected in the T-PCs. Cells were allowed to ingest FBTs for 5 min before fixation. Actin was stained with phalloidin (green) and myosin II was labeled using anti–pMyosin II-LC2 (ser19) antibodies (white). Insets are single z-planes from framed regions. Bars, 5 µm. (B) Myosin II activity is not needed for FBT internalization. 20 min after initial attachment of FBTs, external segments of FBTs were labeled (blue) in the cold. Cells were treated with blebbistatin and phagocytosis allowed to proceed for 20 min in its presence. External FBTs were labeled (green). Number of FBTs undergoing internalization after the treatment was quantified. Data shown are means ± SEM from three independent experiments. At least 25 FBTs were analyzed in each case.

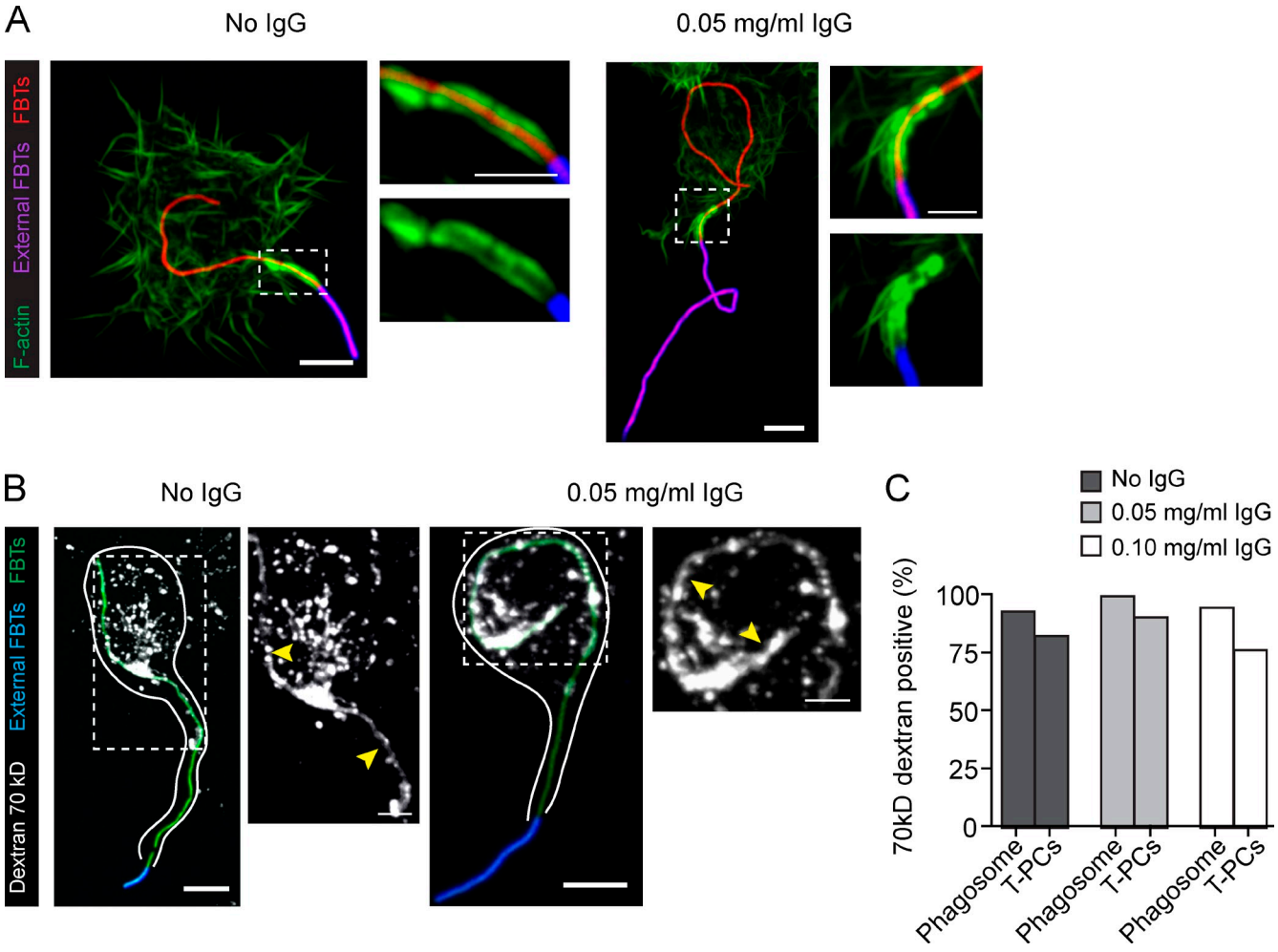
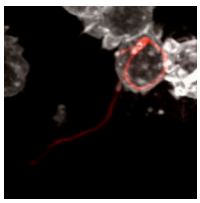
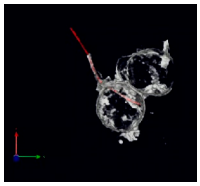


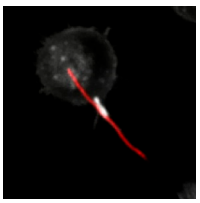
Figure S5. **T-PC and T-PC barrier formation are opsonin-independent processes.** RAW cells were presented either with un-opsonized FBTs or FBTs opsonized with 0.05 mg/ml IgG and phagocytosis was performed in absence of serum. (A) T-PC labeled with F-actin, formed after 20 min of phagocytosis. Actin was stained with phalloidin (green) and external FBTs were immunolabeled (pink). Panels to the right are higher magnifications of framed regions, showing the actin jackets. (B) T-PC barriers are independent of opsonins. Cells preloaded with 70-kD rhodamine dextran were allowed to ingest unopsonized or unopsonized FBTs for 20 min. Cells were fixed and external bacteria were labeled (blue). Higher magnification of framed regions is shown to the right. White lines indicate cell boundaries. Arrowheads point to dex70kD accumulation in the T-PCs. (C) The number of T-PCs from B that retained dex70kD were enumerated. Data shown are percentages from a representative experiment out of two independent experiments. For the data shown, $n = 40$. Bars: (main panels) 5 µm; (magnifications) 2.5 µm.



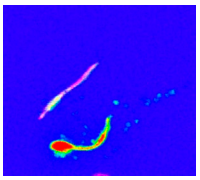
Video 1. **Phagocytosis of FBT by RAW cells stably expressing GPI-GFP.** RAW cells stably expressing GPI-GFP (white) were presented with FBTs (red). Attachment was synchronized and cells were then moved to pre-warmed microscope stage (37°C/5% CO_2). 15 min after the onset of phagocytosis (T0), images were analyzed by time-lapse confocal microscopy using a spinning disc confocal microscope and EM-CCD camera (Quorum Technologies Inc.). Frames were acquired at a rate of 1/min. Images shown are merged, deconvolved z- planes and the movie is played at the rate of 5 frames per second. Related to Fig. 1.



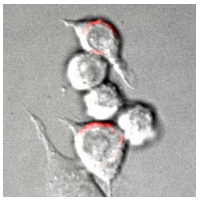
Video 2. **3D rendering of a T-PC holding a FBT.** RAW cells stably expressing GPI-GFP (white) were allowed to internalize FBTs (red) for 10 min. Cells were fixed with 4% PFA and external FBTs were immunolabeled (blue, not depicted). Confocal z-stacks were acquired 0.3 µm apart, deconvolved, and 3D rendered using Volocity software (PerkinElmer) to visualize a T-PC holding an FBT. Related to Fig. 1.



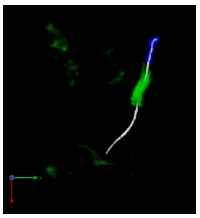
Video 3. **Phagoyctosis of FBT by RAW cells stably expressing GFP-actin.** Live-cell video microscopy showing phagocytosis of FBT (red) by RAW cells stably expressing GFP-actin (white). FBT attachment was synchronized and cells were moved to pre-warmed microscope stage (37°C/5% CO_2). 5 min after initial attachment, images were acquired every minute using a spinning disc confocal microscope and EM-CCD camera (Quorum Technologies Inc.) until complete internalization of the FBT. Images are merged, confocal z-planes. The movie is played at the rate of 5 frames per second. Related to Fig. 2.



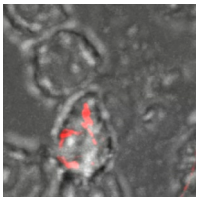
Video 4. **Phagocytosis of DQ-ova–labeled FBT by RAW macrophages.** RAW cells were allowed to phagocytose FBTs (pseudocolored in rainbow) cross-linked with DQ-ova for a 3-h period. Cells were moved to a pre-warmed microscope stage and images (z-stacks) were acquired every hour for a 10-h period using a spinning disc confocal microscope and EM-CCD camera (Quorum Technologies Inc.). Destruction of the FBTs became evident as the target lost its morphology and was degraded. This was accompanied by the lysosomal hydrolysis of the DQ-ova, which caused the bacteria and the debris to fluoresce (green). The movie is played at the rate of 5 frames per second. Related to Fig. 4.



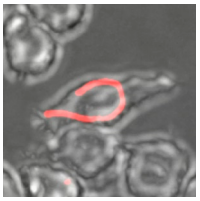
Video 5. **Degradation of intracellular FBTs by RAW macrophages.** RAW cells were allowed to phagocytose FBTs (red) for a 3-h period, followed by live-cell video microscopy using a spinning disc confocal microscope and EM-CCD camera (Quorum Technologies Inc.). Images were acquired every hour for a 10-h period. Frames shown are merged confocal z-stacks. Destruction of the FBTs became evident as the targets lost their morphology and were converted in debris. The DIC image in the first frame shows the macrophages with the intracellular FBTs. The movie is played at 5 frames per second. Related to Fig. 4.



Video 6. **3D rendering showing the constriction of *Salmonella*-FBT by the actin jacket.** RAW cells were presented with PFA-fixed RFP-expressing *Salmonella* filaments (see Materials and methods). Cells were fixed 10 min after initial attachment and external S-FBT segments were immunolabeled (blue) before cell permeabilization to label F-actin with phalloidin (green). Confocal z-planes were acquired 0.3 µm apart using a spinning disc confocal microscope (Quorum Technologies Inc.) and 3D rendered using Volocity software (PerkinElmer). Related to Fig. 7.



Video 7. **Replication of long Lp02 filaments inside RAW macrophages.** RAW cells were infected with Lp02. 10 h after initiation of phagocytosis, external bacteria were immunolabeled and cells were moved to a pre-warmed microscope stage. Infected cells were identified and intracellular survival of individual bacteria assessed by time-lapse live-cell video microscopy using a spinning disc confocal microscope and EM-CCD camera (Quorum Technologies Inc.). Long Lp02 filaments replicated inside RAW cells, leading to cell lysis and escape of bacillary progeny. Images were acquired every 45 min for 15 h and movie is played at 5 frames per second. Related to Fig. 9.



Video 8. **Destruction of intracellular filamentous Lp02 by RAW cells.** RAW cells were infected with Lp02. 5 h after initial attachment external bacteria were immunolabeled and cells were moved to a pre-warmed microscope stage. Infected cells were identified and intracellular survival of individual bacteria assessed by time-lapse live-cell video microscopy using a spinning disc confocal microscope and EM-CCD camera (Quorum Technologies Inc.). Images shown depict an example of intracellular filamentous Lp02 being destroyed by RAW cells. Images were acquired every 45 min for 10 h and movie is played at 5 frames per second. Related to Fig. 9.