Supporting Information

Mesoporous Silica Nanoparticles with pH – Sensitive Nanovalves for Delivery of Moxifloxacin Provide Improved Treatment of Lethal Pneumonic Tularemia

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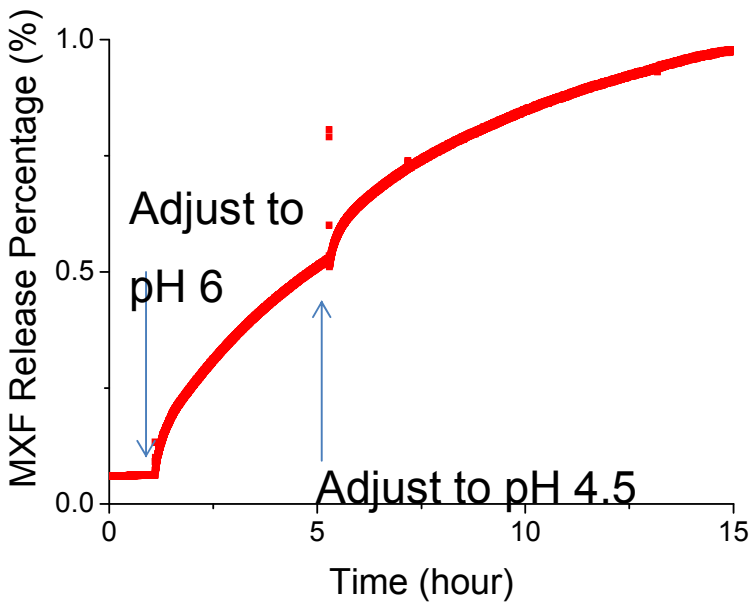

Figure S1. MSN-MBI-MXF release profile. There is no leakage at pH 7 evidenced by the flat baseline. Drug release starts at when the pH is lower than 6. The release rate can be further increased by lowering pH to 4.5.

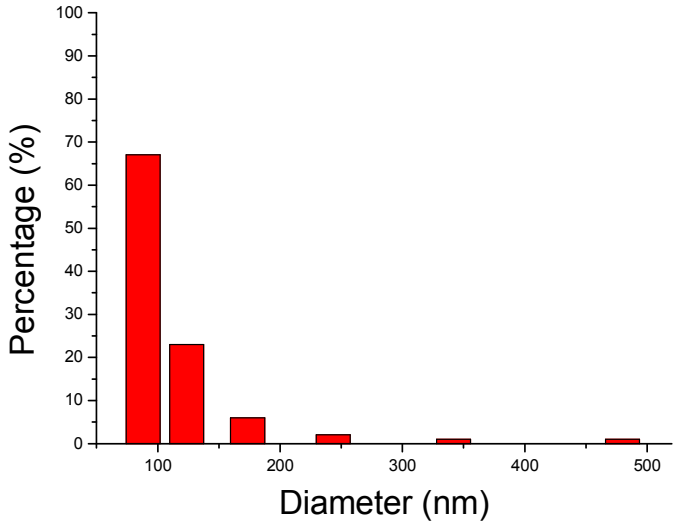

Figure S2. Dynamic light scattering (DLS) measurement of MSN with pH sensitive nanovalve. The mean hydrodynamic diameter of the modified nanoparticle is around 100 nm.

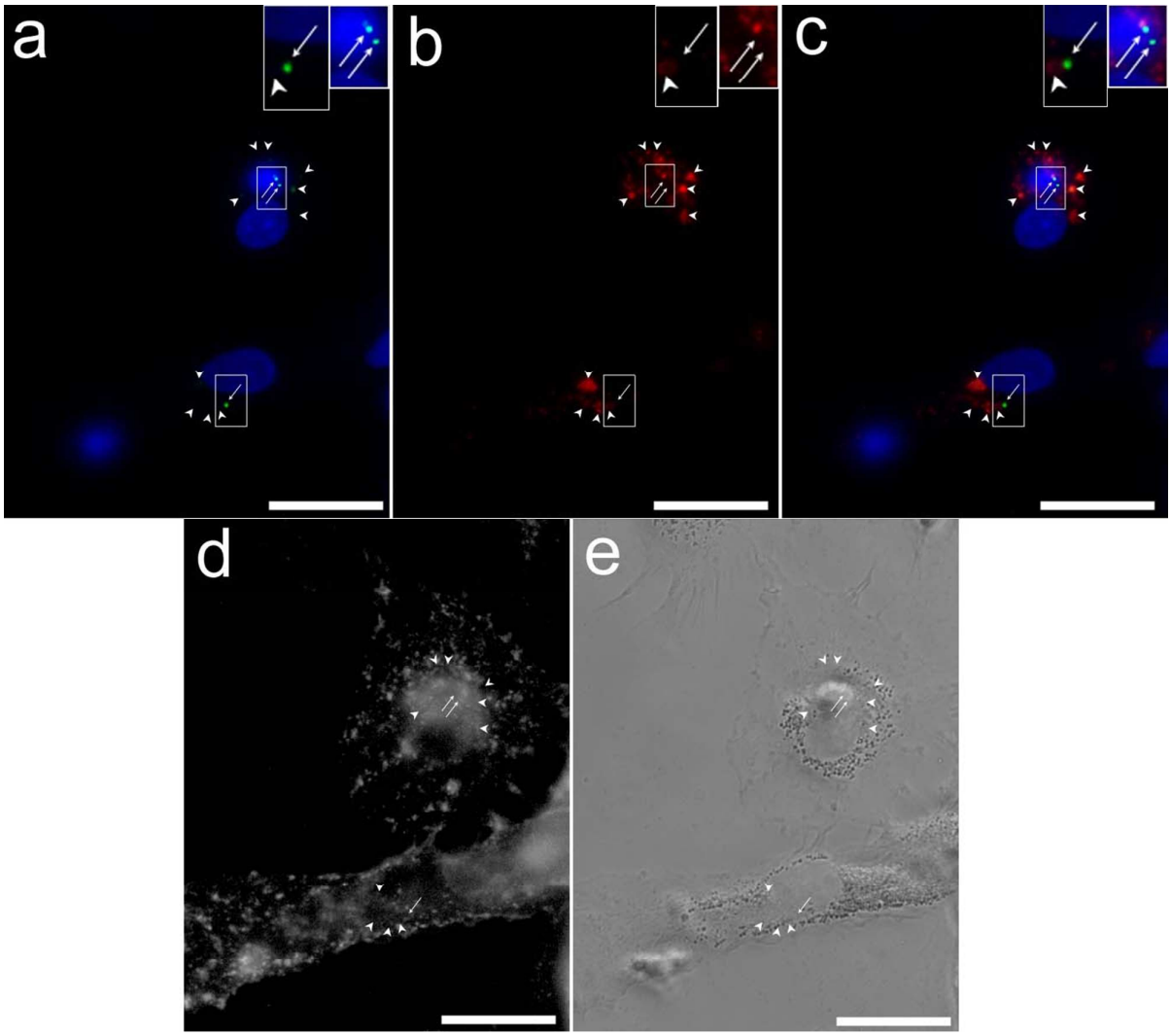

Figure S3. Epifluorescence and phase contrast microscopy demonstrates uptake of RITC-labeled MSN-MBI by *F. tularensis*-infected human monocyte derived macrophages (MDM). Human MDM cells were infected with GFP-expressing *F. tularensis* for 90 min, washed, and incubated with 12.5 μg/mL of RITC-labeled 100 nm MSN-MBI. After 3 hours, the cells were washed; the plasma membrane was stained with WGA-AlexaFluor 633; the cells were fixed; and nuclei were stained with DAPI. (a) LVS-GFP (green, arrows) and DAPI-stained nucleus (blue); (b) RITC-labeled MSN-MBI (red, arrowheads); (c) merged red, green, and blue color

image; (d) contours of the cell are stained with WGA-AlexaFluor 633 (gray scale); (e) phase contrast image. Scale bars, 10 μm. Boxed areas with arrows indicating locations of green fluorescent bacteria are shown at 2-fold higher magnification in the insets in the upper right of panels (a) – (c). The two bacteria overlying the DAPI stained nucleus appear turquoise rather than green because of merging of the blue and green channels. The experiment was done twice with similar results.

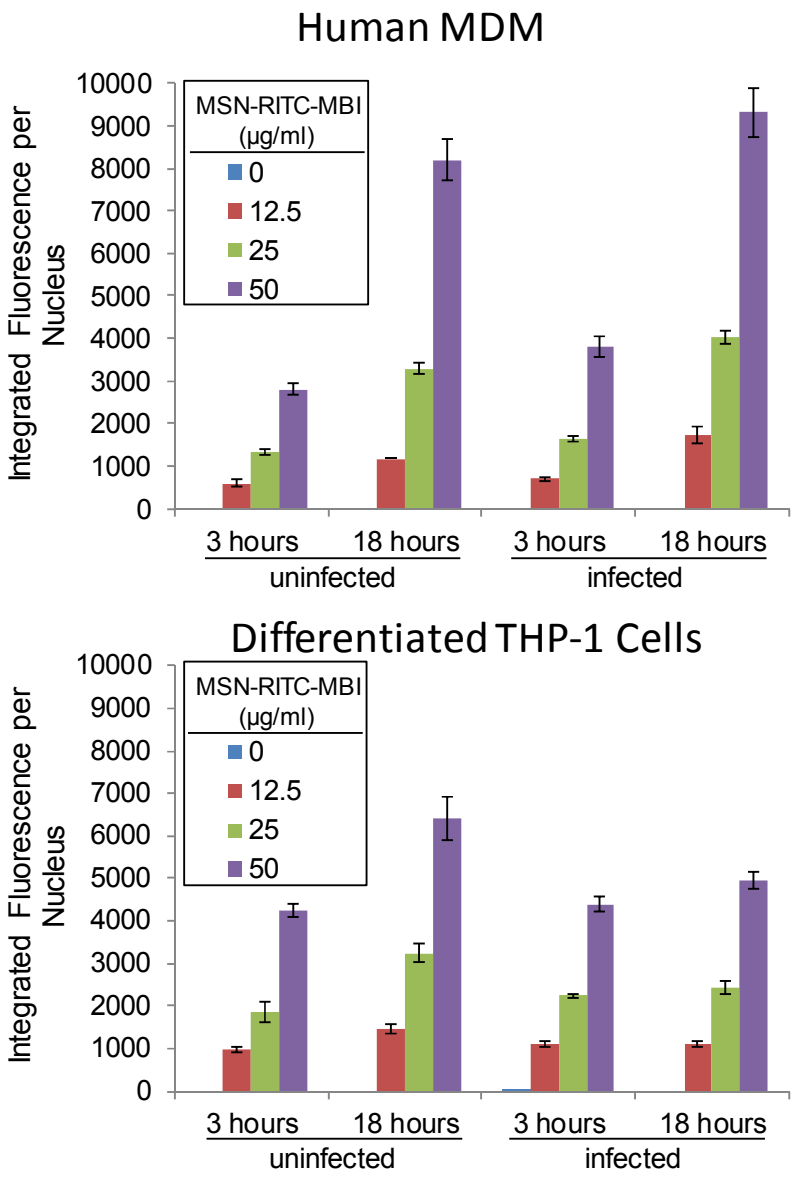

Figure S4. Quantitation of uptake of MSN-RITC-MBI by infected and uninfected human MDM and differentiated THP-1 cells. Uninfected or LVS-GFP infected human MDM (top panel) or THP-1 cells (bottom panel) were incubated with 0, 12.5, 25, or 50 µg/ml of MSN-RITC-MBI for 3 hours or 18 hours prior to staining and fixation as described in Figure S3. Automated high content imaging was performed with an ImageXpress robotic fluorescence microscope and MSN-RITC-MBI

integrated fluorescence intensity per DAPI-stained nucleus was quantitated using the granularity module of MetaXpress software. Data shown represent the means ± S.E. of duplicate wells (Human MDM) or of quadruplicate wells (THP-1 cells). The experiment was performed twice with similar results.

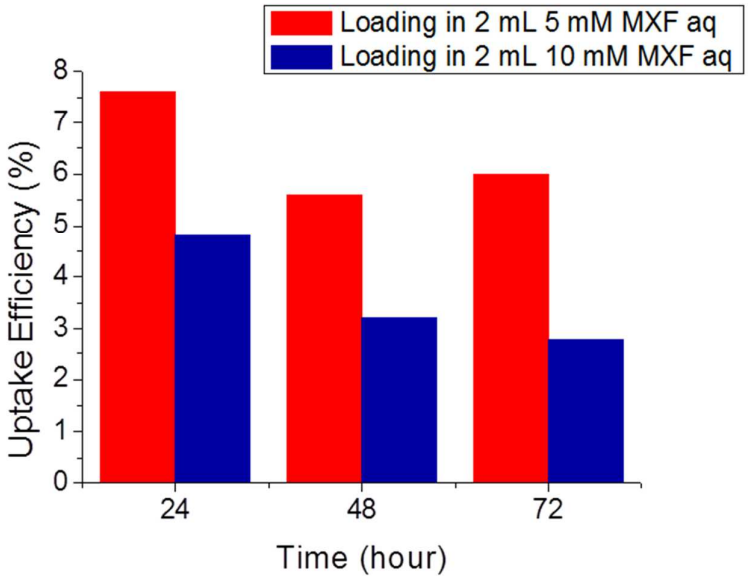

Figure S5. Uptake efficiency of MSN-MBI-MXF loading with 5 mM and 10 mM MXF aqueous solution for 24, 48 and 72 hours. 24 hours loading yielded the highest uptake efficiency for both low and high MXF concentrations.

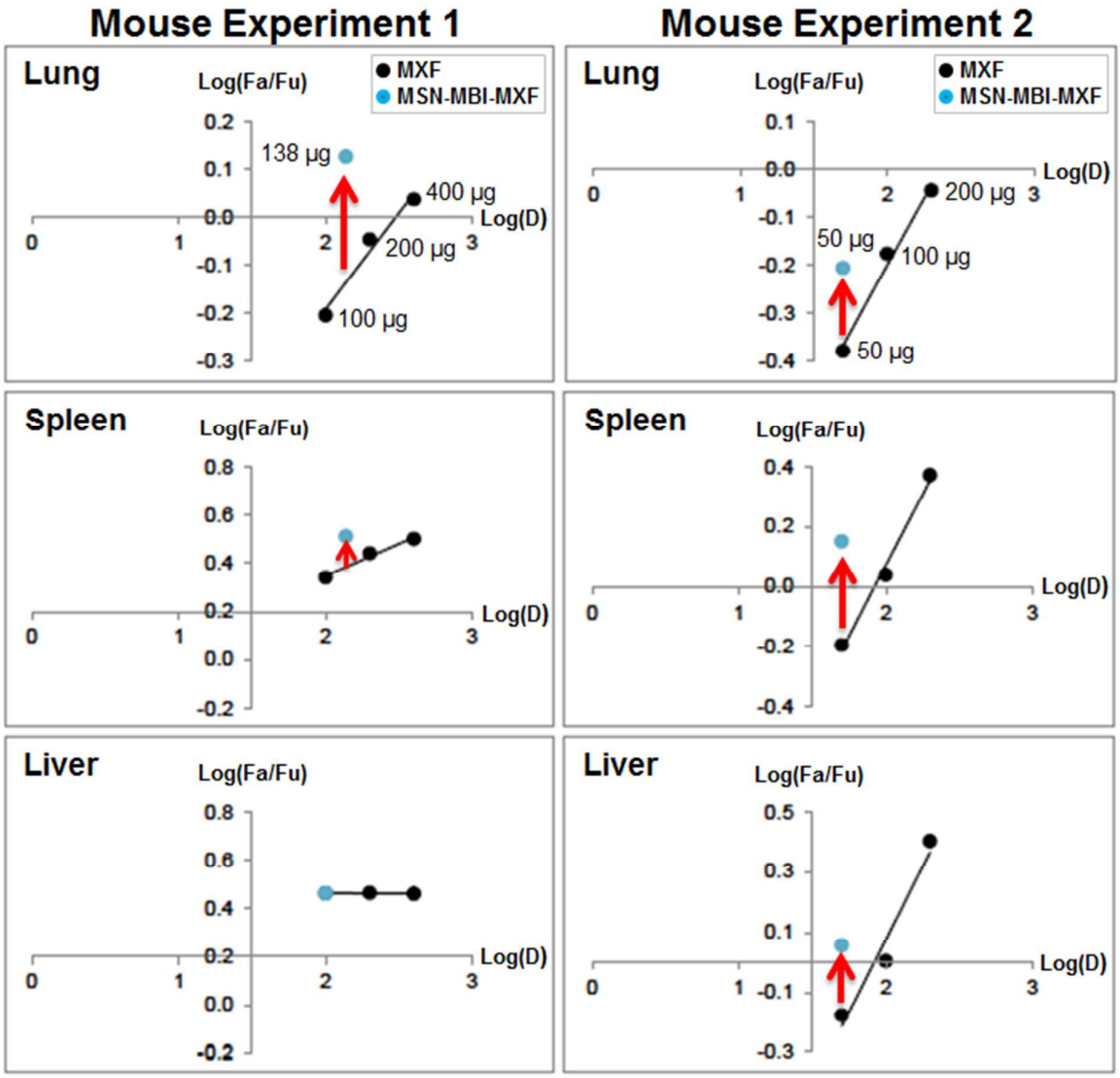

Figure S6. Median-effect plots to compare efficacy of MSN-MBI-MXF with MXF administered as free drug. The efficacy of MSN-MBI-MXF in the lung, spleen, and liver was compared with that of free MXF in a median-effect plot of the results of mouse Experiments 1 and 2. For a given dose of MXF, an upward shift as indicated by the red arrows on the y-axis indicates greater *F. tularensis* killing efficacy of the MSN-MBI-MXF. Fa: Fraction of bacteria killed; Fu: Fraction of bacteria surviving; D: Dose of MXF in micrograms.

Table S1. Bacterial CFU in infected macrophages with and without treatment

Condition	MXF amount	Duration	Log CFU
No treatment	0 ng/ml	3 hours	5.06
No treatment	0 ng/ml	1 day	7.60
MXF	1 ng/ml	1 day	7.65
MXF	2 ng/ml	1 day	7.59
MXF	4 ng/ml	1 day	7.59
MXF	8 ng/ml	1 day	6.92
MXF	16 ng/ml	1 day	5.09
MXF	32 ng/ml	1 day	3.72
MXF	64 ng/ml	1 day	3.43
MSN-MBI control (8 μg/ml)	0 ng/ml	1 day	7.72
MSN-MBI-MXF (0.0625 μg/ml)	1.65 ng/ml	1 day	7.59
MSN-MBI-MXF (0.125 μg/ml)	3.3 ng/ml	1 day	7.21
MSN-MBI-MXF (0.25 μg/ml)	6.6 ng/ml	1 day	5.49
MSN-MBI-MXF (0.5 μg/ml)	13.2 ng/ml	1 day	4.49
MSN-MBI-MXF (1 μg/ml)	26.4 ng/ml	1 day	4.23
MSN-MBI-MXF (2 μg/ml)	52.8 ng/ml	1 day	3.49
MSN-MBI-MXF (4 μg/ml)	105.6 ng/ml	1 day	3.49
MSN-MBI-MXF (8 μg/ml)	211.2 ng/ml	1 day	1.73
MSN-ANA control (8 μg/ml)	0 ng/ml	1 day	7.70
MSN-ANA-MXF (0.0625 μg/ml)	0.23 ng/ml	1 day	7.71
MSN-ANA-MXF (0.125 μg/ml)	0.45 ng/ml	1 day	7.72
MSN-ANA-MXF (0.25 μg/ml)	0.9 ng/ml	1 day	7.59
MSN-ANA-MXF (0.5 μg/ml)	1.8 ng/ml	1 day	7.59
MSN-ANA-MXF (1 μg/ml)	3.6 ng/ml	1 day	7.43
MSN-ANA-MXF (2 μg/ml)	7.2 ng/ml	1 day	7.06
MSN-ANA-MXF (4 μg/ml)	14.4 ng/ml	1 day	5.34
MSN-ANA-MXF (8 μg/ml)	28.8 ng/ml	1 day	3.61