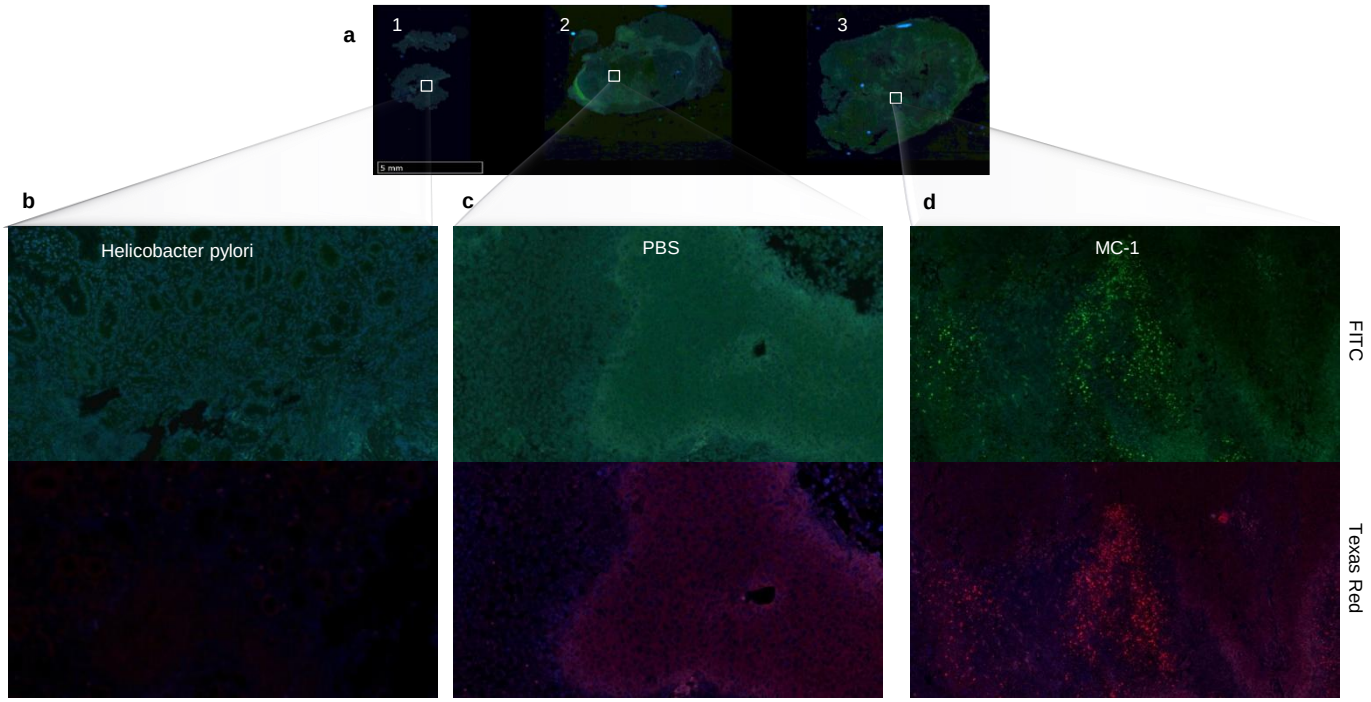
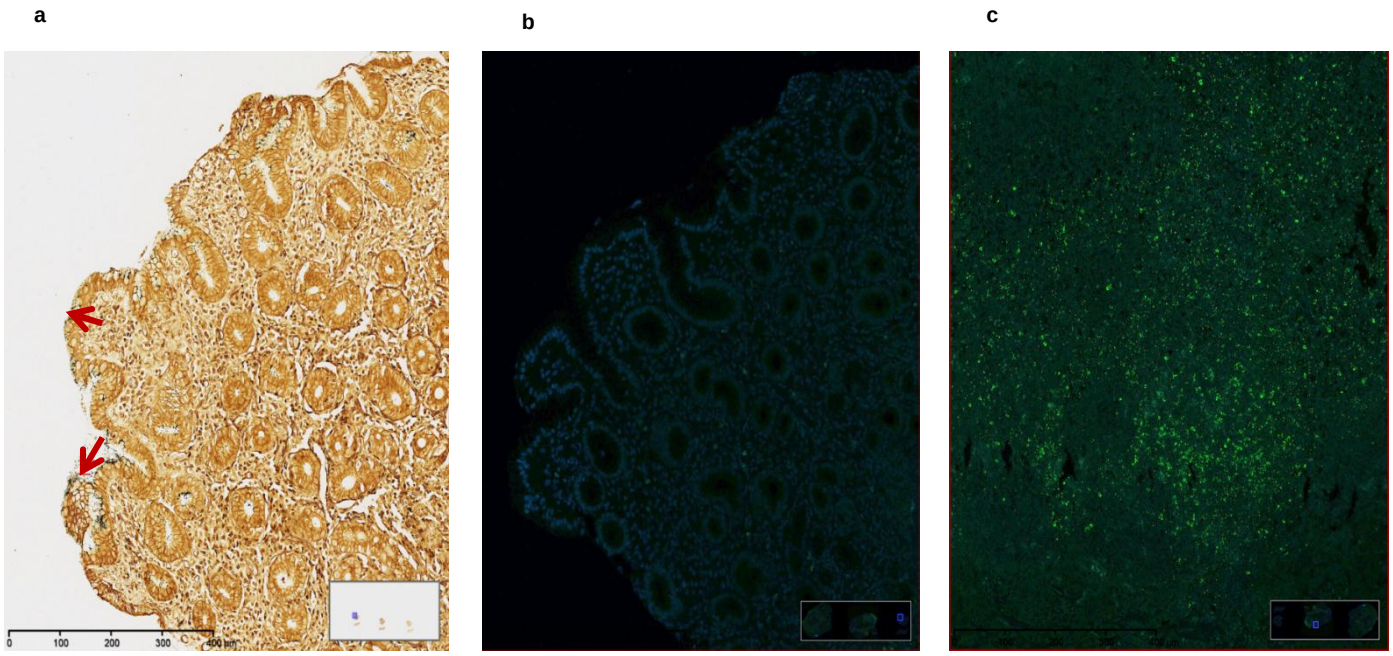
Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to tumour hypoxic regions

Ouajdi Felfoul, Mahmood Mohammadi, Samira Taherkhani, Dominic de Lanauze , Yong Zhong Xu , Dumitru Loghin , Sherief Essa, Sylwia Jancik , Daniel Houle , Michel Lafleur , Louis Gaboury , Maryam Tabrizian, Neila Kaou , Michael Atkin , Té Vuong , Gerald Batist , Nicole Beauchemin , Danuta Radzioch , and Sylvain Martel

Supplementary Figure 1. To reliably detect MC-1 bacteria in the tumours



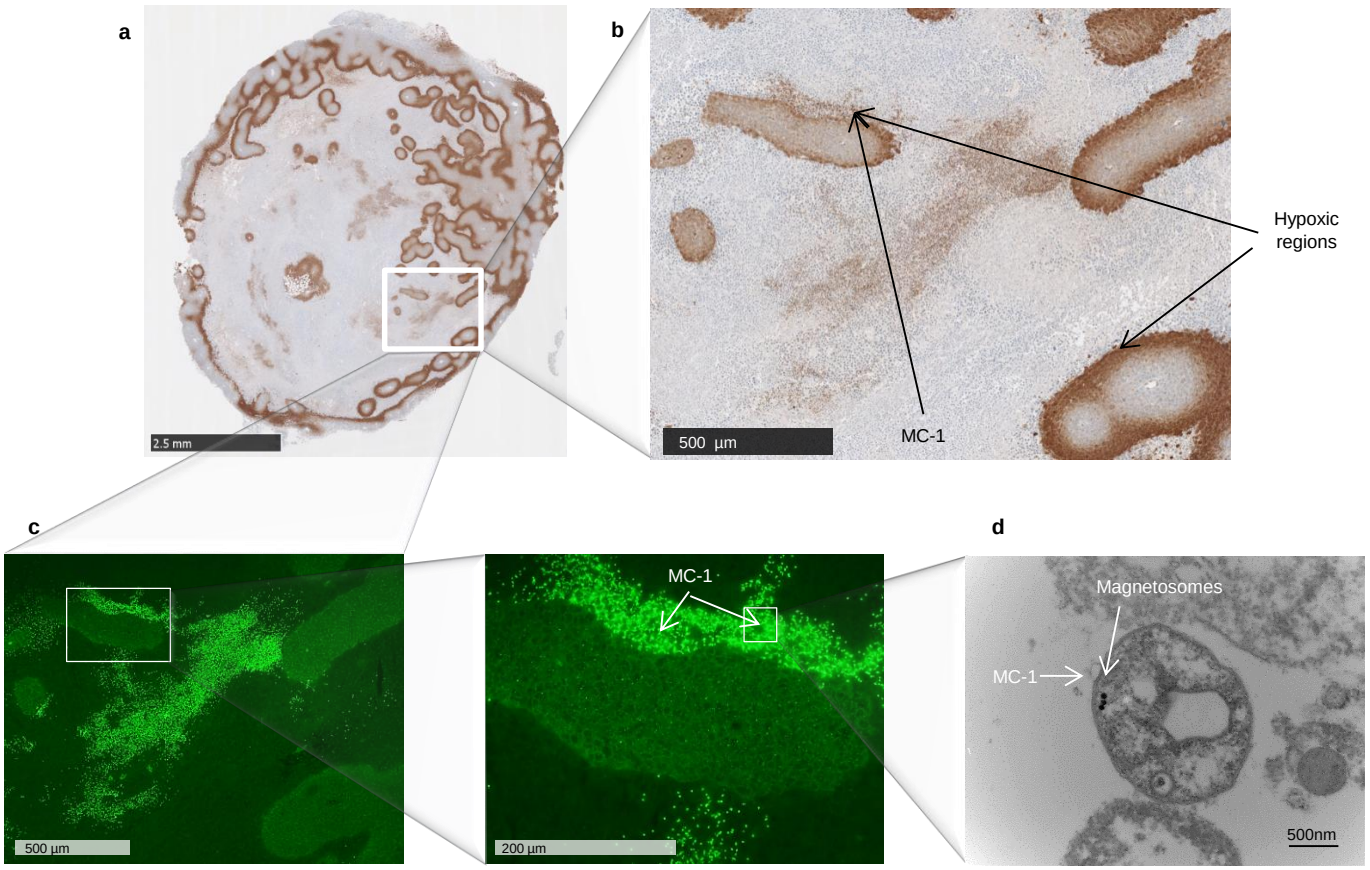
Supplementary Figure 1A. **Assessment of MC-1 antibody specificity using gastric mucosa and xenografts injected with either PBS or MC-1**. **a**, Section of gastric mucosa heavily populated with *Helicobacter pylori* (1), xenograft injected with PBS(2), and xenograft injected with MC-1 (3) were deposited on the same slide. All tissue sections were incubated with the MC-1 antibody. **b**, Section of gastric mucosa chronically infected with *Helicobacter pylori* and incubated with the MC-1 antibody. The reaction was revealed using either FITC (upper panel) or Texas Red (lower panel). No specific labelling was found in the gastric mucosa. **c**, Xenograft injected with PBS and incubated with MC-1 antibody. Absence of specific labelling in the hypoxic areas using either FITC (upper panel) or Texas Red (lower panel) conjugated antibodies. **d**, Xenograft injected with MC-1 and labelled with MC-1 antibody. Both fluorophores (FITC & Texas Red) readily indicate the presence of MC-1 in tumours.



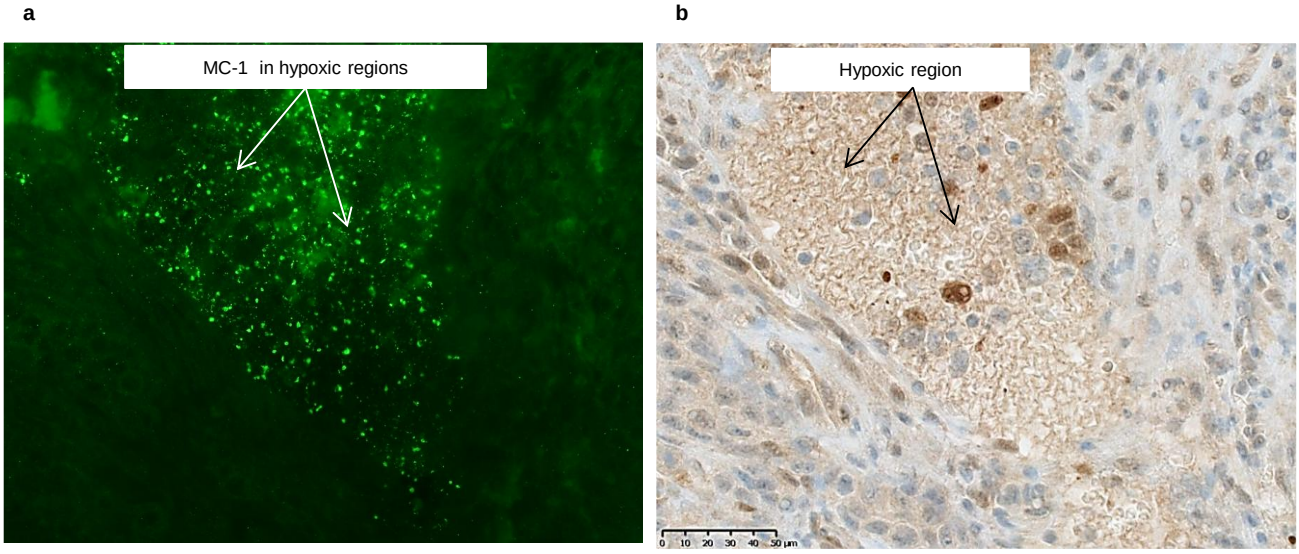
Supplementary Figure 1B. **Assessment of MC-1 specificity**. **a**, Gastric mucosa densely populated with *Helicobacter pylori*, a common pathogen associated with gastritis and gastric ulcer (Whartin-Starry stain*). The *Helicobacter* microorganisms appear on silver staining as small, curved rods on the surface of the gastric mucosa. This section was used as a specificity control for the MC-1 antibody. **b, c**, Section of gastric mucosa heavily populated with *Helicobacter pylori* (b) was deposited on the same slide next to a section of xenograft injected with MC-1 (c). Both tissue sections were incubated with the MC-1 antibody. No specific labelling was observed with *Helicobacter pylori* (b). Section of a xenograft incubated with MC-1antibody shown to be teeming with clusters of MC-1 (c).

*Histotechnology –A self-instructional Text. 3rd ed. FL Carson & C Hladik editors. American Society for Clinical Pathology Press. (2009) p. 244.

Supplementary Figure 2. Hypoxic regions



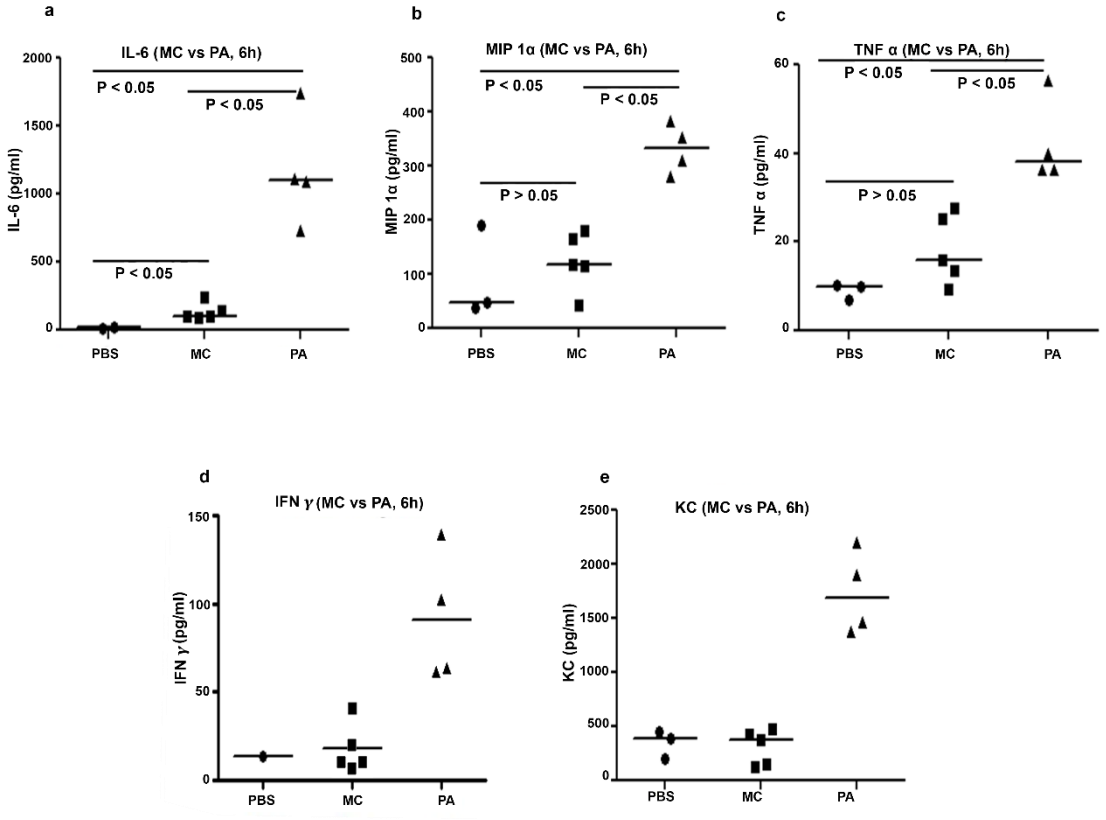
Supplementary Figure 2A. **MC-1 cells are preferentially located in the hypoxic regions of xenografts.** To determine the exact location of MC-1 with regards to the local oxygen tension in tissue, we took advantage of the hypoxyprobe specific antibody (a and b) and the MC-1 antibody that specifically labelled MC-1. **a, b**, strands and islands of remaining hypoxic tumour tissue give a positive brownish reaction in the vicinity of necrotic areas. **c**, Adjacent sections of the same xenografts were incubated with MC-1 antibody and a FITC conjugated specific secondary antibody to label MC-1. We next extracted samples from the paraffin embedded material for TEM. **d**, the identity of the MC-1 cell was confirmed according to their typical ultrastructural features (magnetosomes).



Supplementary Figure 2B. **MC-1 cells accumulate in hypoxic regions of xenografts.** MC-1 labelled section (**a**) confirms the presence of abundant MC-1 in areas of lowered oxygen concentration as shown by the hypoxic probe reaction and the confirmatory histologic signs of oxygen deprivation such as ghosts of cells and poorly stained residual nuclei (**b**).

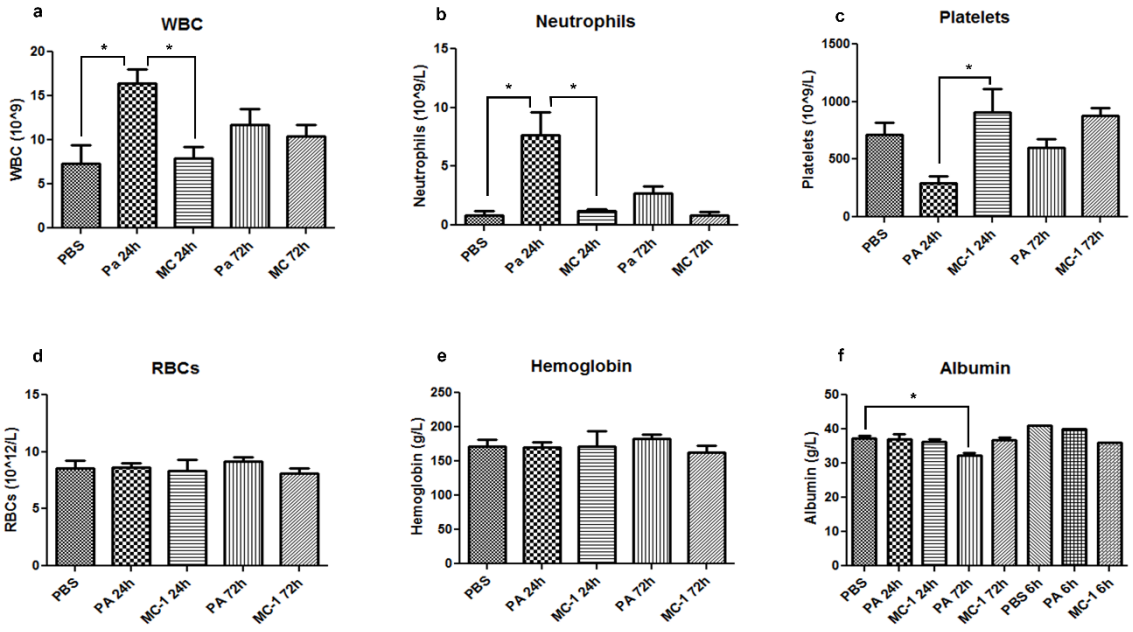
Supplementary Figure 3. Cytotoxicity

MC-1 did not affect the levels of inflammatory cytokines produced in mice, while the positive control *Pseudomonas aeruginosa (PA)* produced significant increases in inflammatory cytokines relative to the negative control (Vehicle, PBS) and relative to positive control (PA)



Supplementary Figure 3. Injection of MC-1 does not induce inflammatory cytokine increases in mice. C57BL/6 mice were injected with 10^7 MC-1, *PA*, or PBS. Blood was harvested at 6 h post injection, and analyzed via ELISA on Luminex to assess the levels of inflammatory cytokines. * means $p<0.05$. A) IL-6 levels in MC-1 infected mice are similar to the control, while significantly elevated in *PA* infected mice. B) MIP1α levels in MC-1 infected mice are similar to the control, while significantly elevated in *PA* infected mice. C) There is not a significant increase in TNFα level in MC-1 infected mice compared to the negative control. D) IFN-γ levels in MC-1 infected mice are similar to the control, while significantly elevated in *PA* infected mice. E) KC levels in MC-1 infected mice are similar to the control, while significantly (*, $p<0.05$) elevated in *PA* infected mice. (Bars show means ± SEM (*$p\leq0.05$, *).

Supplementary Figure 4. Cytotoxicity



Supplementary Figure 4. MC-1 do not alter blood cell counts when injected into rats. Ten week old Sprague-Dawley rats were injected intravenously in the tail with either 150 µL of PBS, or 150 µL 10^8 MC-1 or *Pseudomonas aeruginosa (PA)* resuspended in PBS. *PA* was used as the positive control. Blood was sampled at 6, 24 and 72 h post infection. A) White blood cell counts of rats infected with MC-1 at 24 and 72 h do not significantly differ from the PBS negative control. Rats infected with *PA* have significantly elevated white blood cell counts at 24 h. B) Neutrophil levels in MC-1 infected rats do not differ from the negative control, while there is a spike in *PA* infected rats at 24 h post infection. C) Platelet counts dropped in *PA* infected rats at 24 h post infection, while platelet counts in MC-1 infected rats remain fairly consistent similar to the levels of the PBS control. D) Red blood cell levels remain consistent in the MC-1 infected rats and the positive and negative control. E) Hemoglobin levels also remain constant. F) Albumin levels slightly dip in the *PA*-infected rats at 72 h post infection, while levels remain constant in the MC-1 infected and negative control. Haematological assessment of the blood was performed by McGill McIntyre Medical Services. All animal work was approved by the McGill University Animal Care Committee, in compliance with the Canadian Council of Animal Care guidelines.