

Figure S2

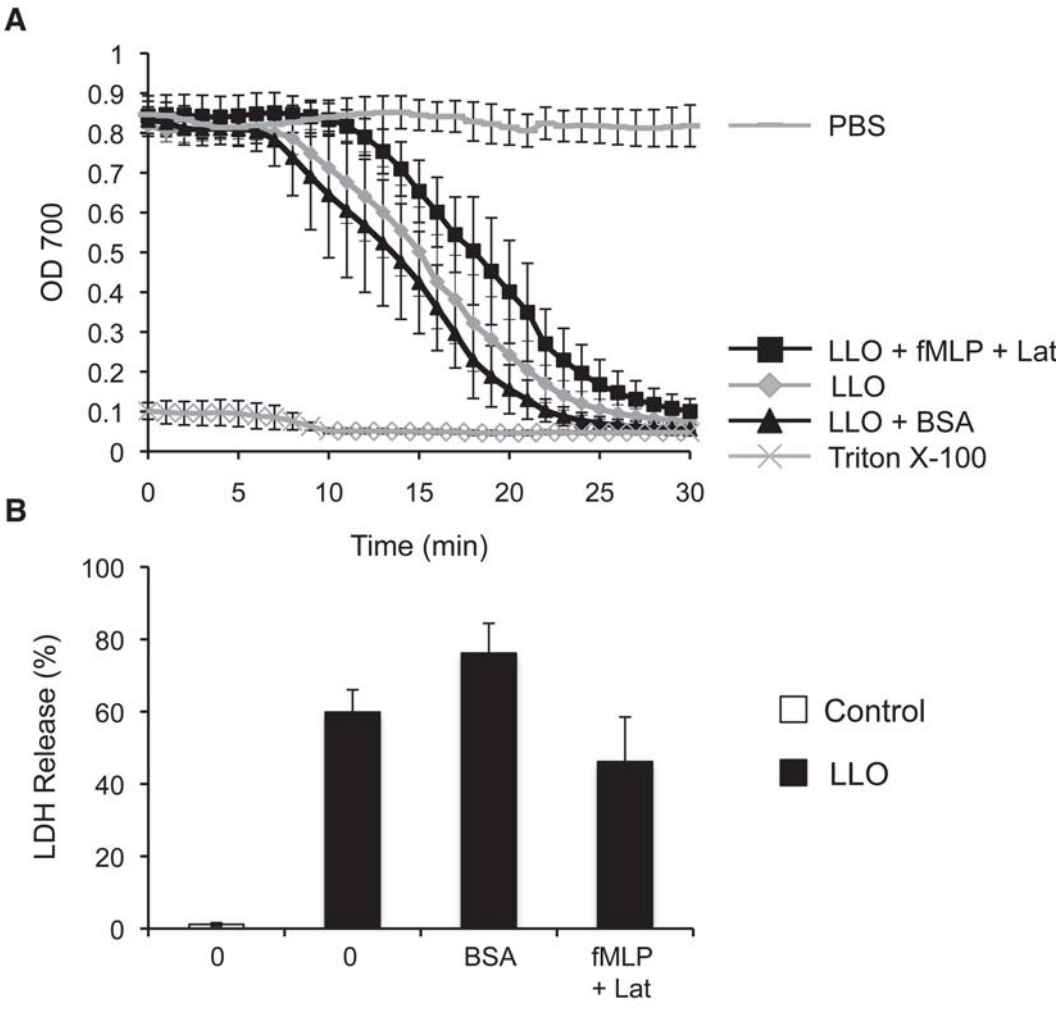
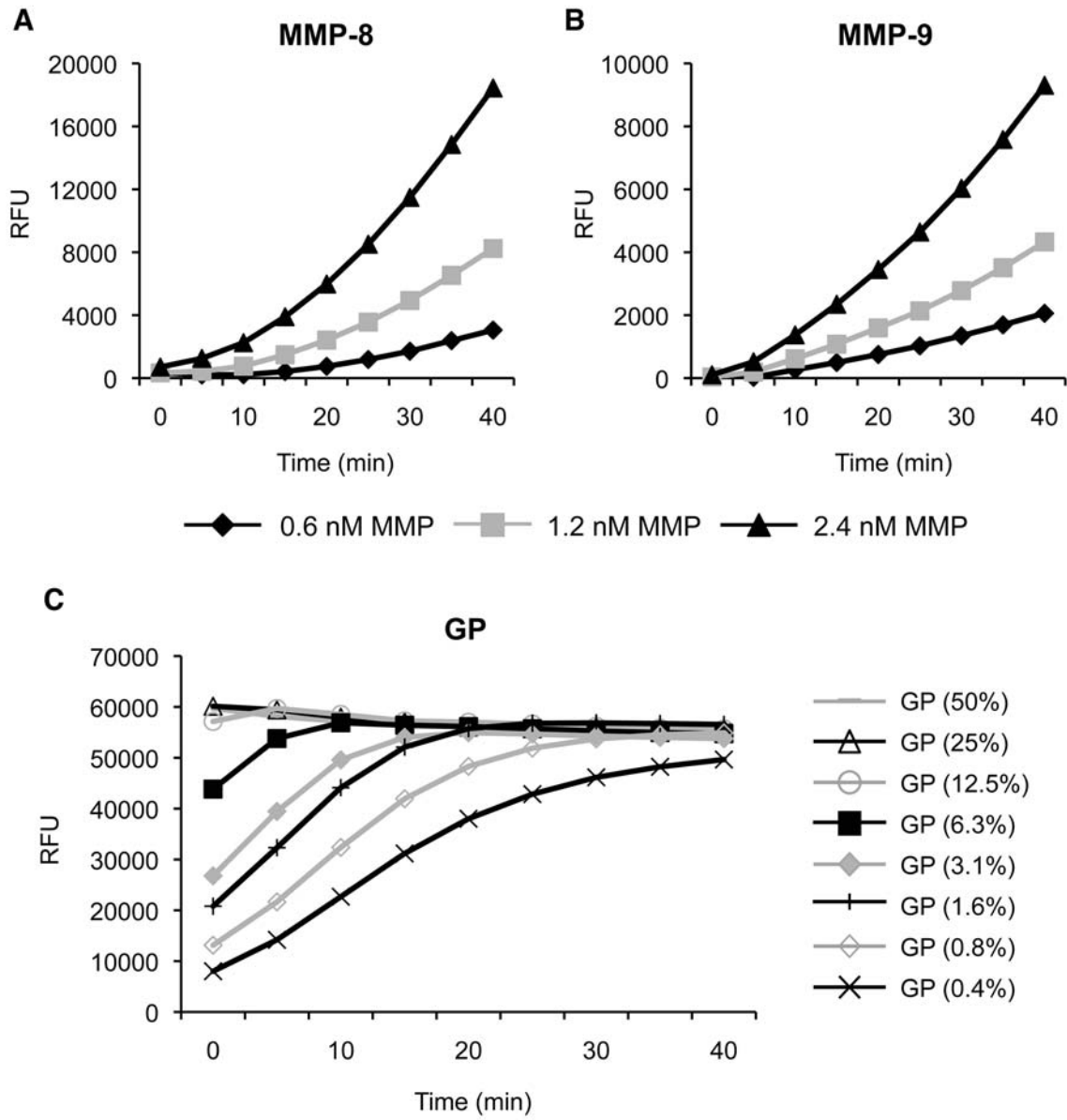


Figure S3



## Supplemental Figure Legends

**Figure S1. LLO-dependent *L. monocytogenes* replication in macrophages.** (A) *L. monocytogenes* (*Lm*; wt or  $\Delta hly$ ) were incubated alone (*Lm*) or with RAW264.7 macrophages (*Lm* + RAW) in 10% Serum/DMEM at 37°C for the indicated times at MOI 1. To measure intracellular bacterial viability (*Lm* + RAW, Intra.), gentamicin was added to the cell culture medium during the last hour of incubation and cells were washed. Triton X-100 was added and cell lysates were plated to enumerate CFUs. Results are expressed relative to the inoculum and are the mean  $\pm$  SEM of at least three independent experiments, performed in triplicate, \*:  $P < 0.05$ . (B) RAW264.7 macrophages were incubated with wt or  $\Delta hly$  *L. monocytogenes* at MOI 1 and 10 in 10% Serum/DMEM for 4 h at 37°C, then 20  $\mu$ M propidium iodide (PrI) was added and cells were incubated for 1 h. Cells were fixed and the MFI of PrI in the nuclear regions was determined by quantitative fluorescence microscopy, as we previously described (11, 12).

**Figure S2. Neutrophil granule products inhibit LLO (additional controls for Fig. 6).** To ensure that fMLP, latrunculin A (Lat), and proteins (BSA) do not directly affect LLO activity, perforation of erythrocytes (A) and RAW264.7 macrophages (B) was measured in the presence or absence of those molecules, at the highest concentration used in Figure 6. (A) Erythrocytes were incubated with 0.24 nM LLO. Triton X-100 (0.05%) was added to induce maximal lysis. Data are expressed as mean OD 700  $\pm$  SEM of at least three independent experiments, each performed in duplicate. (B) Macrophages were incubated with media alone (Control), 5 nM LLO, BSA, or fMLP + Lat for 15 min at 37°C. LDH release is expressed as mean percentage of maximal LDH release  $\pm$  SEM of at least three independent experiments, each performed in duplicate.

**Figure S3. Similar to MMP-8 and MMP-9, neutrophil granule products display MMP activity.** MMP-8 (A), MMP-9 (B), or GP (C) were incubated with 1  $\mu$ M 5-FAM/QXL 520 MMP FRET substrate III (Anaspec, Fremont, CA) in 50 mM Tris-HCl pH 7.6, 200 mM NaCl, 5 mM CaCl<sub>2</sub>, 20  $\mu$ M ZnCl, and 0.05% Brij L23 in 96-well plates. Energy transfer from 5-FAM to QXL 520 quenches the fluorescence emission of 5-FAM, but upon cleavage by MMPs, QXL dissociates from 5-FAM enabling the detection of 5-FAM fluorescence. Plates were incubated for 40 min at 37°C and fluorescence intensity of 5-FAM (485 nm excitation and 528 nm emission) was acquired every 5 min.