# Supplemental Material

## Maresin 1 Biosynthesis and Pro-resolving Anti-Infective Functions with Human Localized Aggressive Periodontitis Leukocytes

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**Supplemental Figure 1.** MaR1 enhances macrophage intracellular ROS generation with *P. gingivalis* and *A. actinomycetemcomitans*. (**A**) Macrophages from healthy donors ($5x10^4$ cells/ well) were incubated with fluorescent carboxy-$H_2$DCF-DA for 30 min before incubation with vehicle ($PBS^{+/+}$, 37°C, pH=7.4) or MaR1 (10 pM- 100 nM) for 15 min followed by addition of bacteria (Mϕ: bacterium; 1:50) for 60 min. Intracellular ROS generation was assessed using a fluorescence plate reader. Results are expressed as percent increase above vehicle; mean± SEM, n=5, *p<0.05, **p<0.01, ***p<0.001 vs. vehicle alone. (**B**) Macrophages ($2x10^4$ cells/ well) from LAP and matched HC were pre-incubated with carboxy-$H_2$DCF-DA for 30 min before incubation (37°C) with vehicle ($PBS^{+/+}$, pH=7.4) or MaR1 (1 nM) for 15 min followed by addition of bacteria (Mϕ: bacterium; 1:50) for 60 min. Intracellular ROS generation was assessed using a fluorescence plate reader. Results are expressed as percent increase above vehicle; mean± SEM, n=5 paired donors, $^{\#\#}$p<0.01, $^{\#\#\#}$P<0.001, HC vs. HC plus vehicle, **p<0.01, ***p<0.001, LAP vs. LAP plus vehicle; † † p<0.01 HC plus bacteria, MaR1 vs. vehicle alone, $^{\S}$p<0.05, $^{\S\S}$p<0.01 LAP plus bacteria, MaR1 vs. vehicle alone. *Inset* is the relative fluorescence unit (RFU) in the absence of bacteria.

**Supplemental Figure 2.** Representative images of the bacterial cultures following incubation of macrophages from HC and LAP patients with vehicle ($PBS^{+/+}$, 37°C, pH=7.4) or MaR1 (1 nM).

**Supplemental Figure 3.** MaR1 enhances neutrophil intracellular ROS generation for *P. gingivalis* and *A. actinomycetemcomitans*. Isolated human PMNs ($10^5$ cells/ well) from

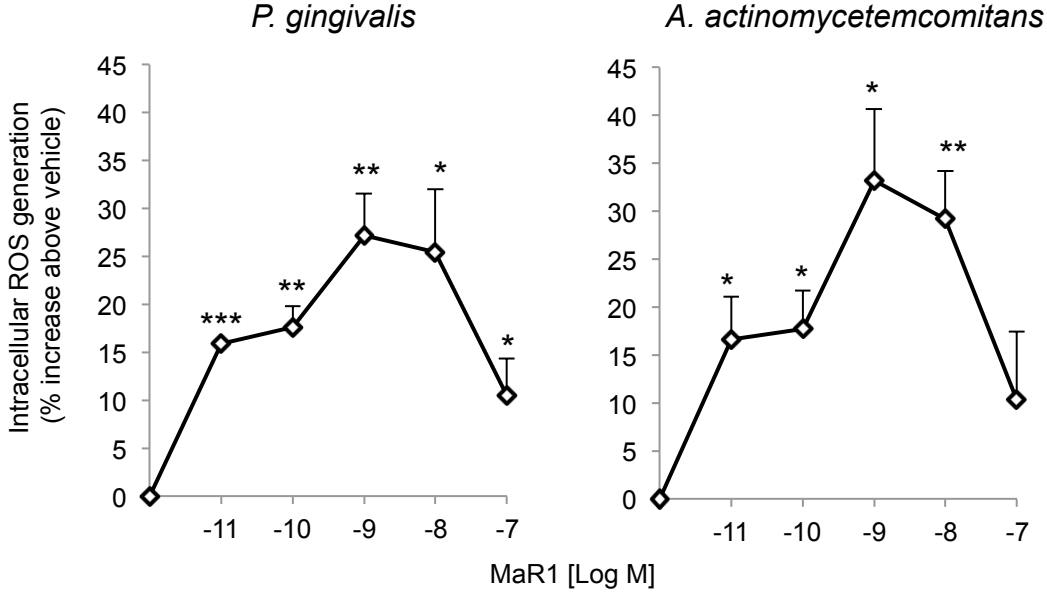
HC and LAP patients were incubated with carboxy-$H_2$DCF-DA for 30 min. Vehicle ($PBS^{+/+}$, 37°C, pH=7.4) or MaR1 (0.1 pM- 100 nM) was then added to PMN for 15 min prior to addition of the bacteria (PMN: bacterium; 1:50) for 60 min. The intracellular ROS production was quantified using a fluorescence plate reader. Results are expressed as percent changes normalized to HC; mean± SEM, n=7, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, HC vs. HC plus vehicle, *p<0.05, **p<0.01, LAP vs. LAP plus vehicle.

**Supplemental Figure 4.** MaR1 enhances macrophage phagocytosis of *F. nucleatum*. Incubation of vehicle ($PBS^{+/+}$, 37°C, pH=7.4) or MaR1 (10 pM- 100 nM) with macrophages from healthy donors ($5x10^4$ cells/ well) for 15 min prior to addition of fluorescence-labeled bacteria (Mϕ: bacterium; 1:50). Phagocytosis was assessed by a fluorescence plate reader. Results are expressed as percent increase above vehicle; mean± SEM, n=4 healthy donors, *p<0.05, **p<0.01 vs. vehicle alone.

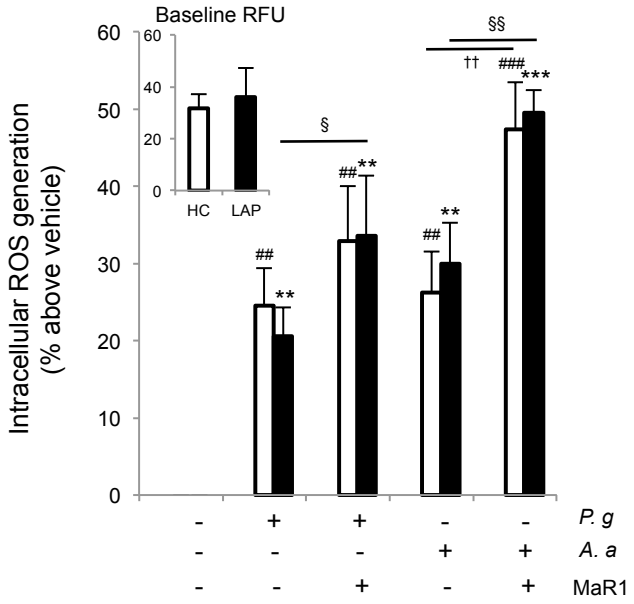
**Supplemental Figure 5.** Maresin 1 levels were reduced after pathogen incubations. (A) Levels of Maresin 1 in macrophage incubations with and without periodontal pathogens were determined using LC-MS-MS based LM-metabololipidomics (see *Methods* for details). Results are expressed as % of vehicle; mean ± SEM, n=4 (B) Representative levels of Maresin 1 in macrophage incubations from one paired HC and LAP patients.

SUPPLEMENTAL FIGURE 1

A

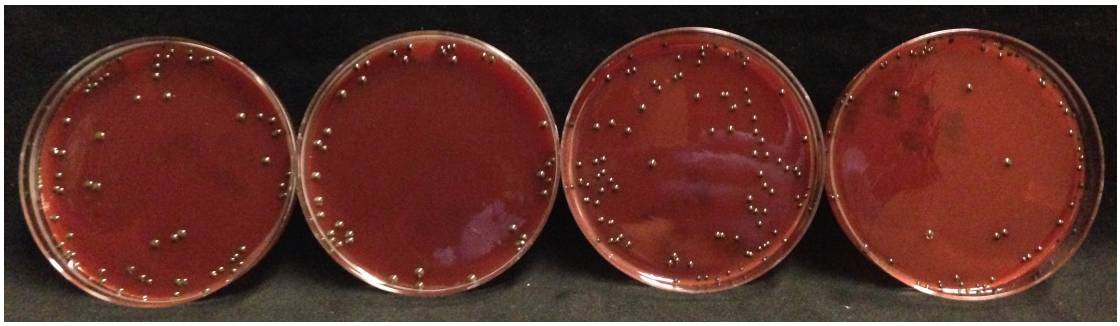



B

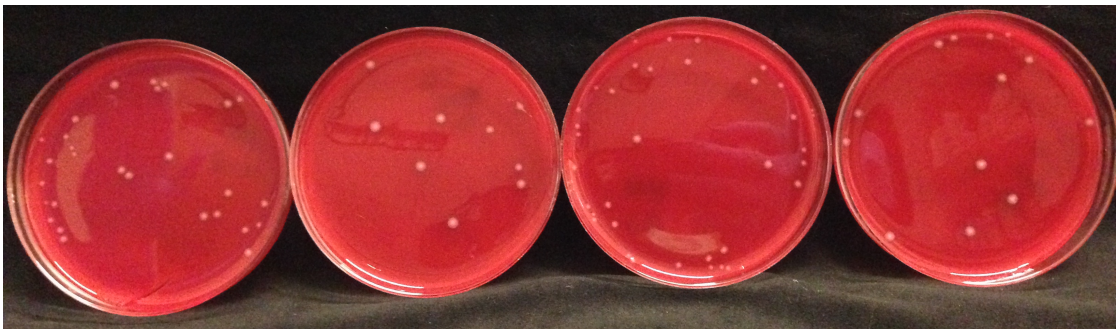


SUPPLEMENTAL FIGURE 2

*P. gingivalis culture*

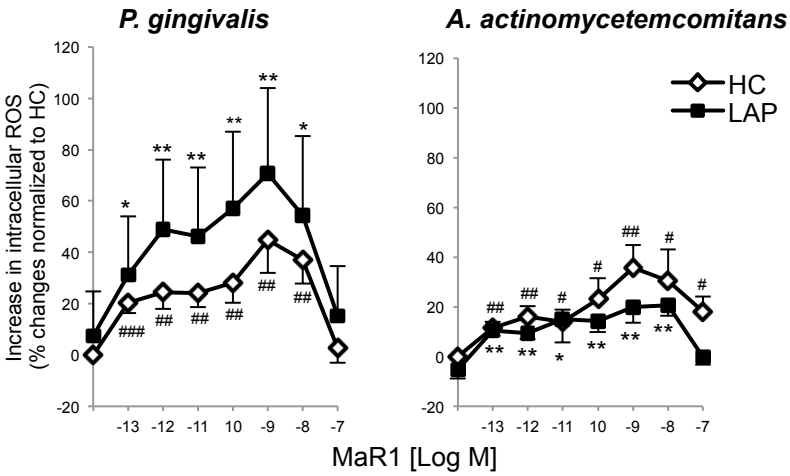


*A. actinomycetemcomitans* culture

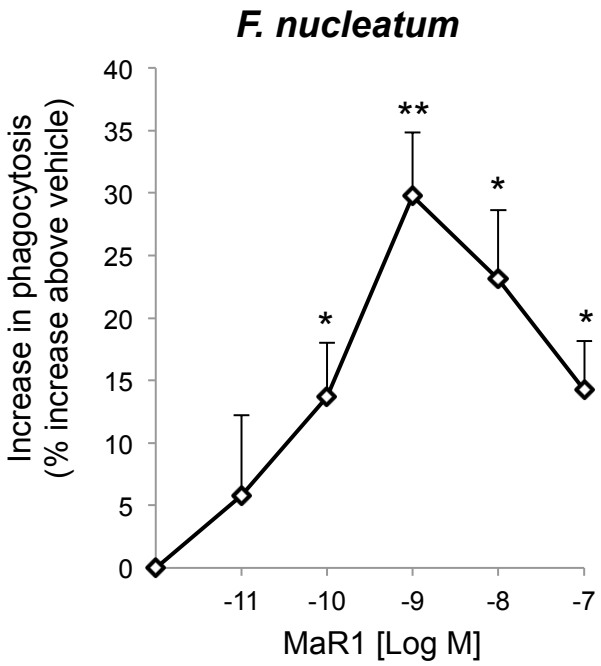


HC HC+MaR1 LAP LAP+MaR1

SUPPLEMENTAL FIGURE 3



SUPPLEMENTAL FIGURE 4



SUPPLEMENTAL FIGURE 5