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Supplemental information

Moraxella catarrhalis evades neutrophil oxidative stress responses providing a safer niche for nontypeable *Haemophilus influenzae*

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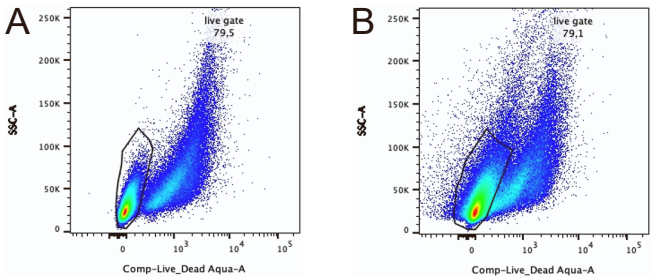


C

	% of viable dHL-60 cells	
	Uninfected	Mcat-infected
Rep 1	76,1	77,4
Rep 2	79,5	79,1
Rep 3	87,7	89,69
Rep 4	86,71	87,81
Rep 5	84,31	85,23

Figure S1: *M. catarrhalis*-mediated ROS suppression is not due to increased cell-death, Related to Figure 2. Representative example of viability (LIVE/DEAD Fixable Aqua Dead Cell Stain) for ROS-induced, (A) non-infected and (B) infected cells (MOI 50) at 75 minutes after infection. (C) Summary data of the percentage of viable cells across multiple replicate experiments.

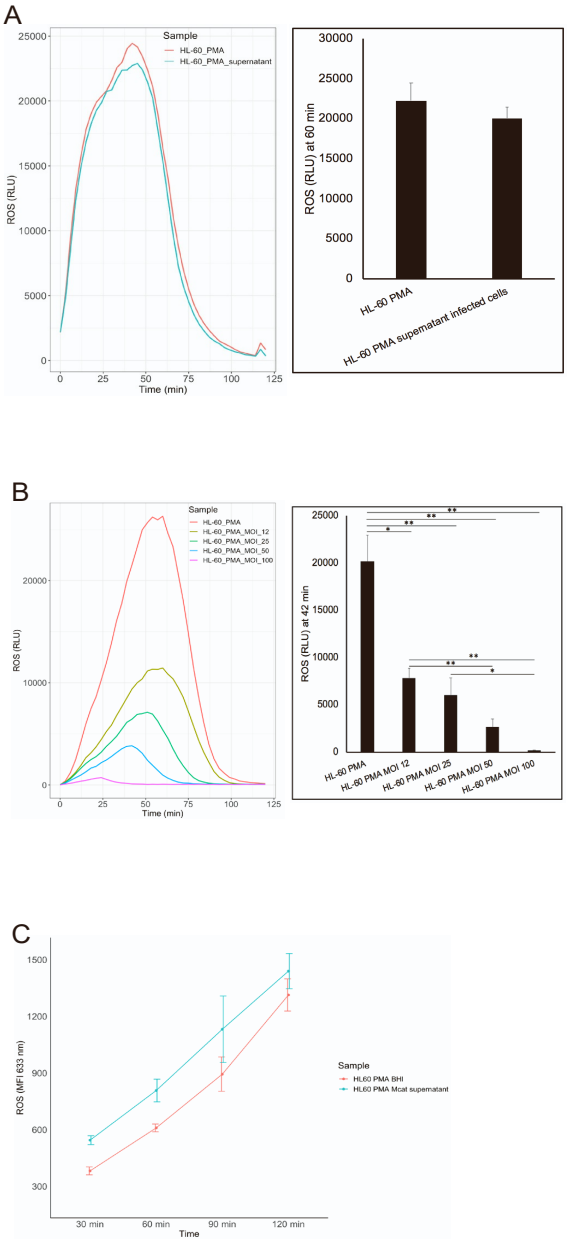


Figure S2: *M. catarrhalis* limits ROS production in a contact-dependent and phagocytosis-independent manner, Related to Figure 3. (A) dHL-60 were infected or not by Mcat BBH18 strain (MOI 50) and the resulting supernatants were presented to naïve ROS-induced cells. (B) ROS-induced dHL60 cells were pre-incubated with cytochalasin D (phagocytosis inhibitor) and infected by Mcat at MOI 100, 50, 25 and 12. (A and B) Representative experiment showing generation of chemiluminescence measured every 3 minutes within 2 hours along with the quantification of ROS at 42 minutes from at least three independent experiments. Bars represent means ± SE. RLU, relative luminescence units. (C) Mcat was grown in BHI to mid-exponential phase and the resulting supernatants or clean BHI (as control) were presented to naïve ROS-induced cells. MFI at 633 nm from at least three independent experiments at the indicated time point after infection was determined by flow cytometry. Bars represent means ± SE. MFI, Mean fluorescence intensity.

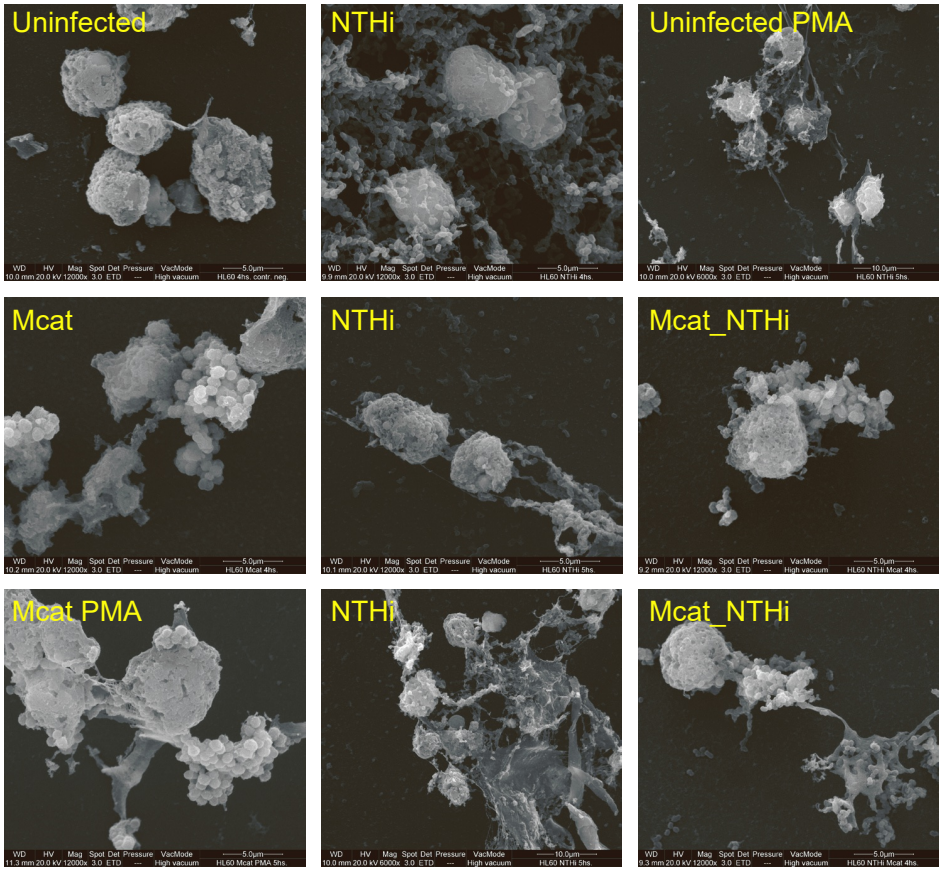
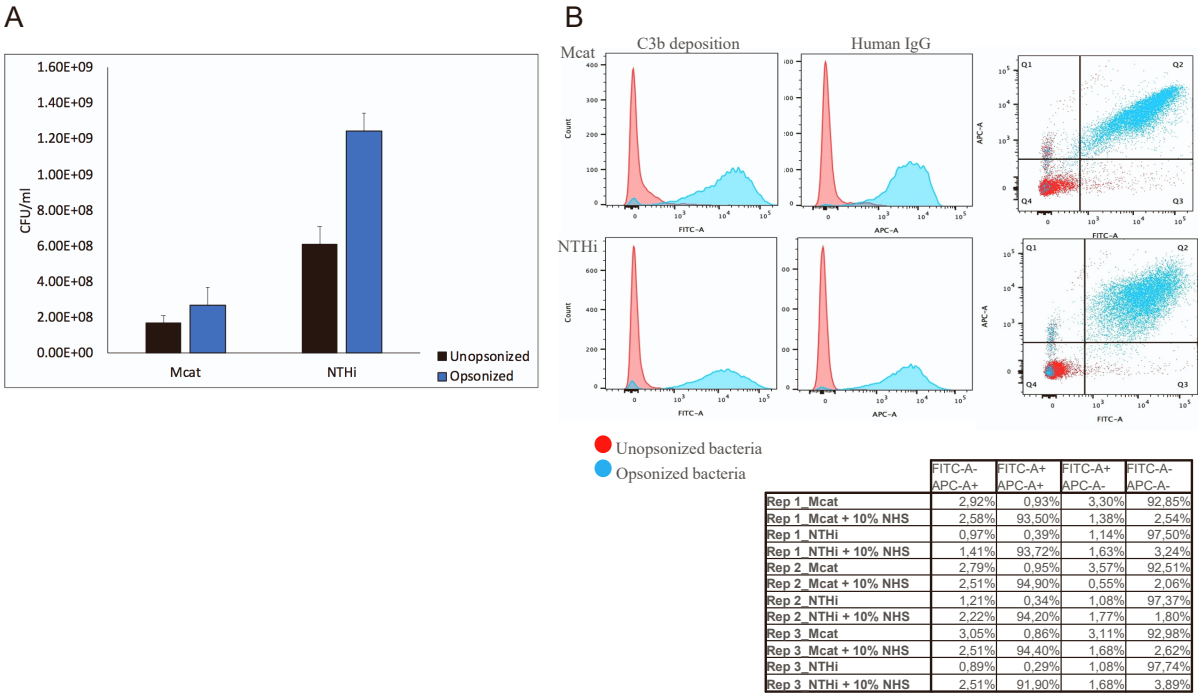


Figure S3: Additional images of NET structures, Related to Figure 4.



	FITC-A- APC-A+	FITC-A+ APC-A+	FITC-A+ APC-A-	FITC-A- APC-A-
Rep 1_Mcat	2,92%	0,93%	3,30%	92,85%
Rep 1_Mcat + 10% NHS	2,58%	93,50%	1,38%	2,54%
Rep 1_NTHi	0,97%	0,39%	1,14%	97,50%
Rep 1_NTHi + 10% NHS	1,41%	93,72%	1,63%	3,24%
Rep 2_Mcat	2,79%	0,95%	3,57%	92,51%
Rep 2_Mcat + 10% NHS	2,51%	94,90%	0,55%	2,06%
Rep 2_NTHi	1,21%	0,34%	1,08%	97,37%
Rep 2_NTHi + 10% NHS	2,22%	94,20%	1,77%	1,80%
Rep 3_Mcat	3,05%	0,86%	3,11%	92,98%
Rep 3_Mcat + 10% NHS	2,51%	94,40%	1,68%	2,62%
Rep 3_NTHi	0,89%	0,29%	1,08%	97,74%
Rep 3_NTHi + 10% NHS	2,51%	91,90%	1,68%	3,89%

Figure S4: Human sera coat bacterial surface with C3b and IgG without affecting their viability, Related to Figure 6. *M. catarrhalis* and NTHi were grown to mid-exponential phase and then treated or not with 10% of pooled human sera. (A) By dilution plating onto BHI or HAE2 plates, the number of colonies obtained per condition (w or w/o NHS) was calculated to plot CFU/ml and to determine the survival of *M. catarrhalis* and NTHi, respectively. (B) Flow cytometry was used to determine C3b and human IgG antibodies deposition on both, unopsonized (red signal) and opsonized (blue signal), Mcat (upper panels) and NTHi (bottom panels). A summary data of the percentage of bacteria binding to C3b (FITC+) and/or human IgG (APC+) across multiple replicate experiments was reported.