

# Non-Thermal Plasma Jet-Treated Medium Induces Selective Cytotoxicity against *Mycobacterium tuberculosis*-Infected Macrophages

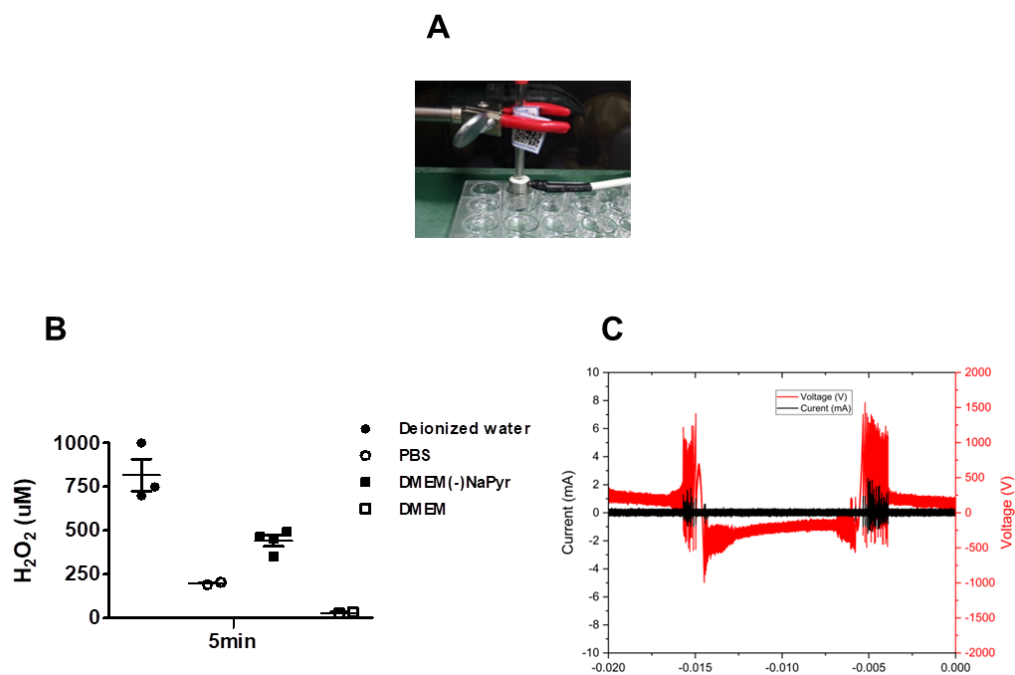
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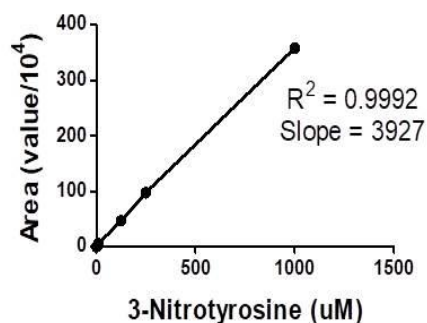
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## Supplemental Figures



**Figure S1.** NTPJ source, H<sub>2</sub>O<sub>2</sub> concentrations at different liquids, and electric power. (A) Photograph of atmospheric pressure jet source. (B) The H<sub>2</sub>O<sub>2</sub> concentration in DW, PBS, DMEM without NaPyr, and complete DMEM for 5 min by NTPJ. (C) Discharge power and voltage-current waveforms of NTPJ at 1.5 SLM (N<sub>2</sub> gas), V= 1.06 kV; I=4.5 mA; P =4.77 W.

A



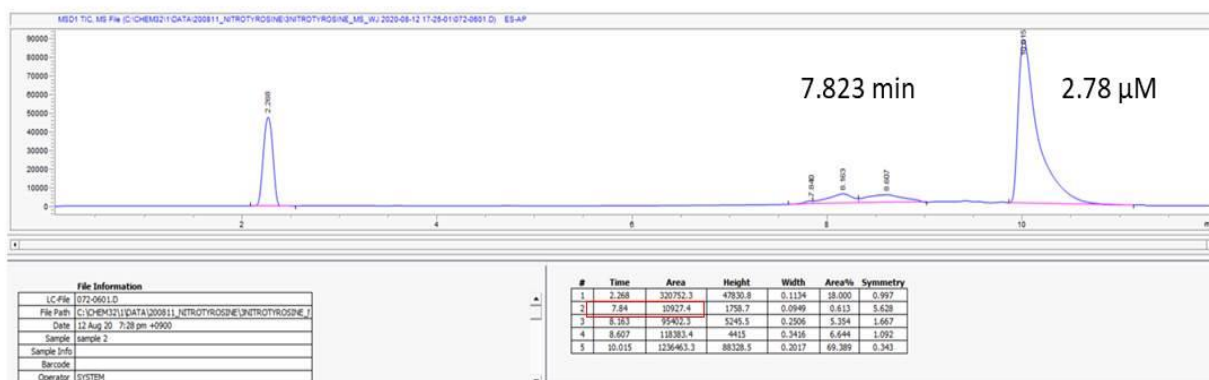
B

## 3-nitrotyrosine

| Conc.    | Time  | Area     | Area%  |
|----------|-------|----------|--------|
| 10 mM    | 7.796 | 20470100 | 90.828 |
| 1 mM     | 7.810 | 3589009  | 63.003 |
| 0.25 mM  | 7.811 | 987528.6 | 37.667 |
| 0.125 mM | 7.811 | 477588.8 | 24.558 |
| 12.5 uM  | 7.851 | 60850.3  | 3.768  |
| 6.25 uM  | 7.840 | 35416.2  | 2.273  |

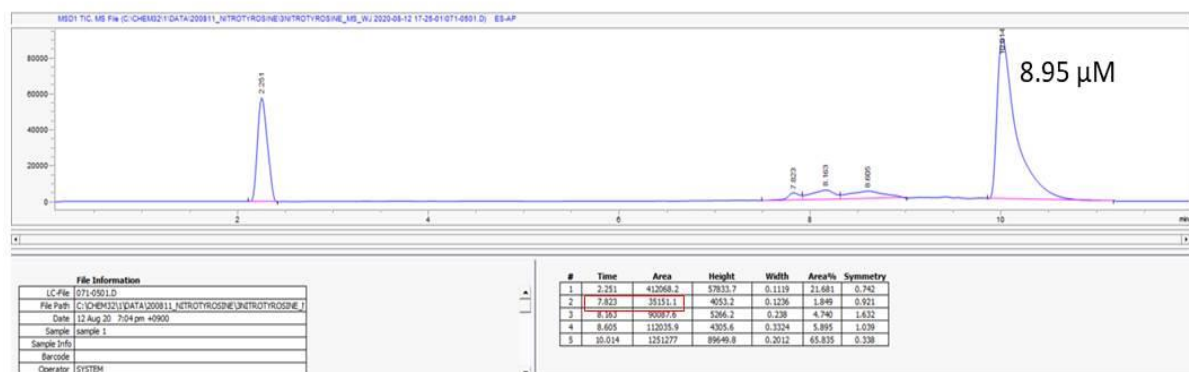
C

## 0.1mM L-tyrosine

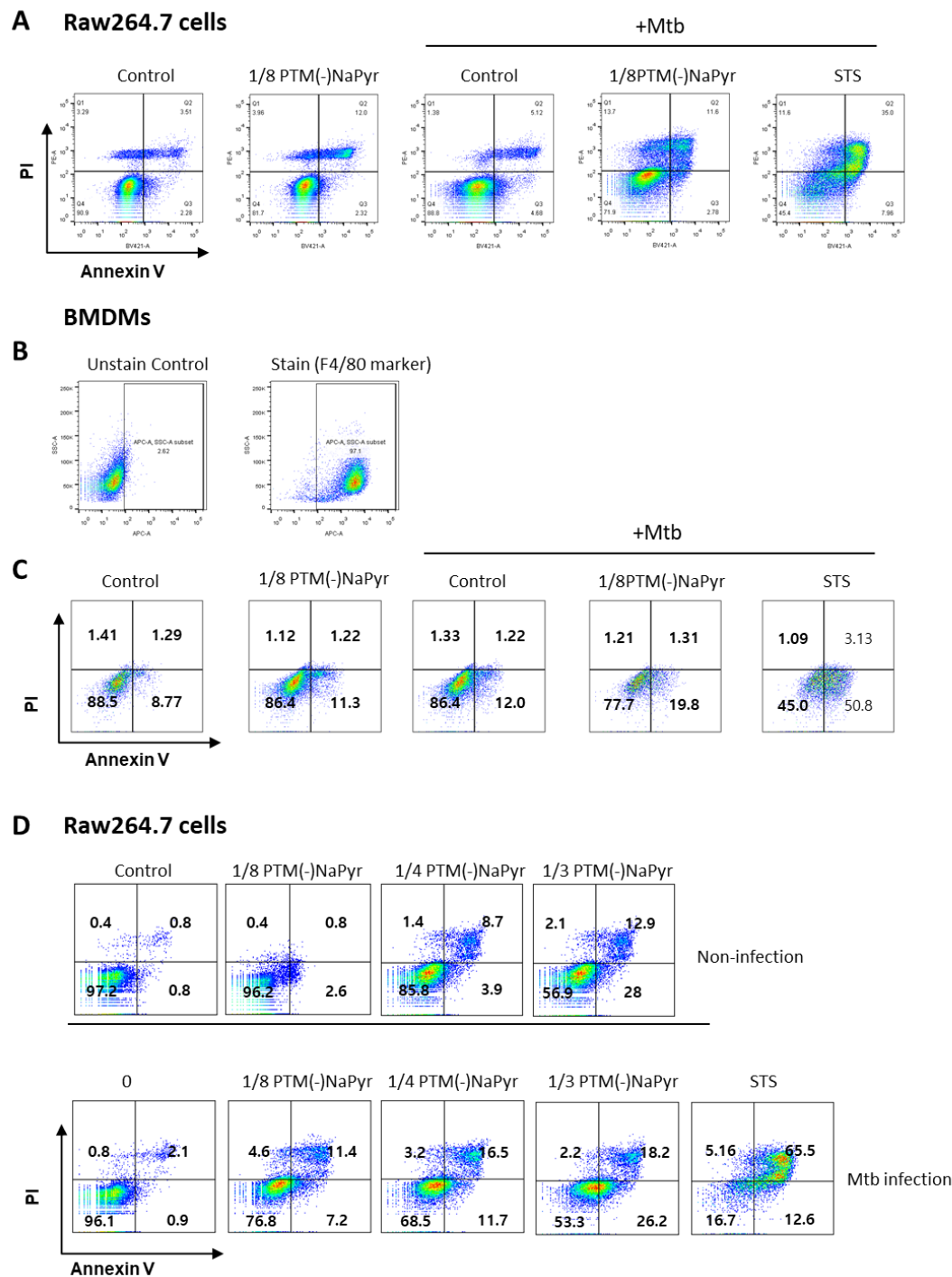


D

## 1mM L-tyrosine

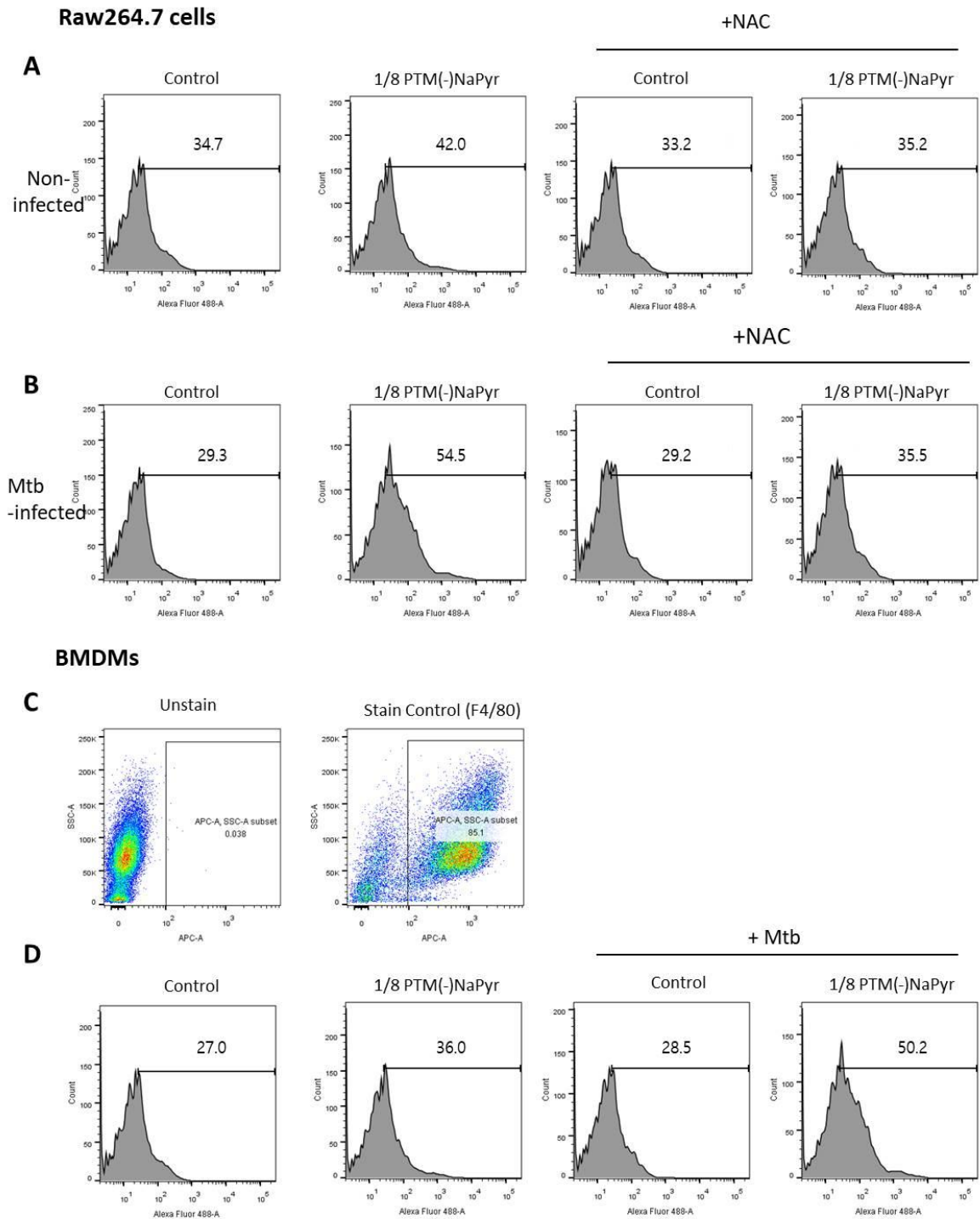


**Figure S2.** ONOO<sup>-</sup> quantification with LC-MS. Calibration curve of 3-NT and 3-NT generation in NTPJ treatment for 5 min. (A-B) Standard graph and area. (C-D) 3-NT value in 0.1mM or 1mM L-tyrosine induced PTM for 5 min treatment, respectively. Retention time: 7.823 min ; 0.1 mM L-tyrosine, Area = 10927; 1 mM L-tyrosine, Area = 35151.

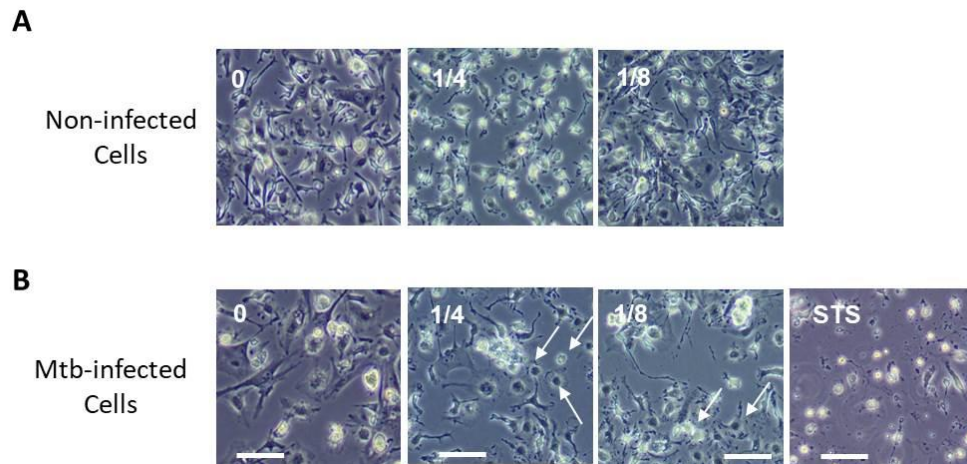


**Figure S3.** Cell death analysis using flow cytometry. (A) *Mtb*-infected Raw264.7 cells (B) Analysis was done by gating live-cell surface marker APC using log phase controls in BMDMs, and STS (50 nM). (C) BMDMs at MOI1 or non-infected cells were treated with 8-fold diluted PTM(-)NaPyr or STS (100 nM).

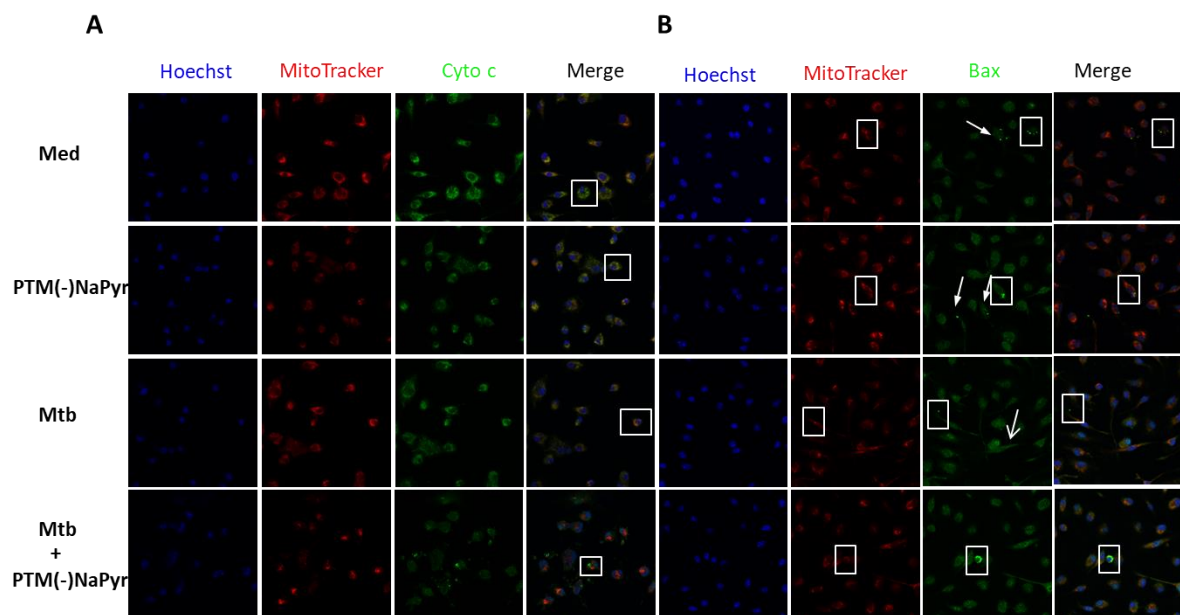
(D) Non-infected and *Mtb*-infected Raw264.7 cells exposed to PTM-diluted medium at different dilution ratios (8-fold, 4-fold, and 3-fold dilution).



**Figure S4.** Intracellular ROS was measured using flow cytometry, stained with H<sub>2</sub>DCFDA.(A-B) uninfected or *Mtb*-infected Raw 264.7 cells. (C) Analysis was done by gating live-cell surface marker APC using log phase controls in unstained BMDMs (D) Stained BMDM cells after 8-fold dilution PTM(-)NaPyr or with pre-treated NAC (10 mM).



**Figure S5.** Images of activation or apoptosis of uninfected or *Mtb*-infected primary macrophages (BMDMs) at 24 h elapsed from 4- or 8- diluted PTM(-)NaPyr culture, respectively. BMDMs ( $2 \times 10^5$  cells/12 well) were infected with *Mtb* (MO1). Cells were treated with 4- or 8- PTM diluted culture, respectively. STS was used 100 nM. Like STS, 4-diluted or 8-diluted PTM culture showed morphological apoptosis similarly STS. The white arrows indicate the apoptosis of *Mtb*-infected cells. Phase contrast bright field images showing morphological changes: (A) uninfected (B) *Mtb*-infected cells. Scale bars, 10  $\mu$ m.



**Figure S6.** Changes in Cyt c and Bax localization in *Mtb*-infected cells exposed to PTM. (A-B) Confocal microscopy images showing MitoTracker (red) and Cyt c or Bax (green) staining of only medium (Med), PTM(-)NaPyr-uninfected BMDMs, *Mtb*-only infection only cells, and 8-fold diluted PTM(-)NaPyr-treated and *Mtb*-infection cells, for 18 h. (A) Cyt c, or (B) Bax. Scale bar = 10  $\mu$ m.

**Table S1.** Oligonucleotides used to perform qRT-PCR analysis

| Oligonucleotide | Oligonucleotide Sequences (5'-3') |
|-----------------|-----------------------------------|
| RT CAT F        | CACTTTGGAGTTGTGCCGTG              |
| RT CAT R        | TGGCTCGGACCAGATCAGTA              |
| RT ATM F        | AGGGGAGCGAGTGTTTGTAG              |
| RT ATM R        | GCAGCAAGTTGGTAAGGCAC              |
| RT GPx1 F       | CGTGCAATCAGTTCGGACAC              |
| RT GPx1 R       | CCGAGGATAGGCCTGCATTC              |
| RT 18S F        | GTAACCCGTTGAACCCCATC              |
| RT 18S R        | CCATCCAATCGGTAGTAGCG              |