Supplementary Information

Table S1. The concentration of hydrogen peroxide, nitrite, and nitrate were measured using test strips for media containing serum and different cell preparations. All RONS measured were above the detection limit. Evaporation was also calculated by subtracting the weight of the sample after NTP exposure and the weight of the sample before NTP exposure, but little change in weight was measured. Weights ranged from 197 mg to 334 mg.

	pH	Hydrogen Peroxide (ppm)	Nitrite (ppm)	Nitrate (ppm)
Serum-free DMEM	8.2	25–50	>25	>500
Serum + DMEM	7.8	25	>25	>500
Serum	7	25	>25	>500
Live Vero cells	8.1	25	>25	>500
Fixed Vero cells	7.9	25	>25	>500
Freeze–thaw Vero cells	7.9	25	>25	>500
Heat-killed Vero cells	7.9	25	>25	>500

pH, which can be used as an indicator for RONS generation, varied between samples. With the exception of the NTP-killed Vero cells, the pH did not drop below 7 for any of the cell conditions or media with and without serum present. However, killed cells did have lower pH measurements compared to live cells and media alone. The most acidic sample (pH 6.5) was measured for Vero cells killed by high NTP frequencies, which could be indicative of a higher amount of RONS present as result of a greater NTP generation of RONS in media (Supplementary Table 1).

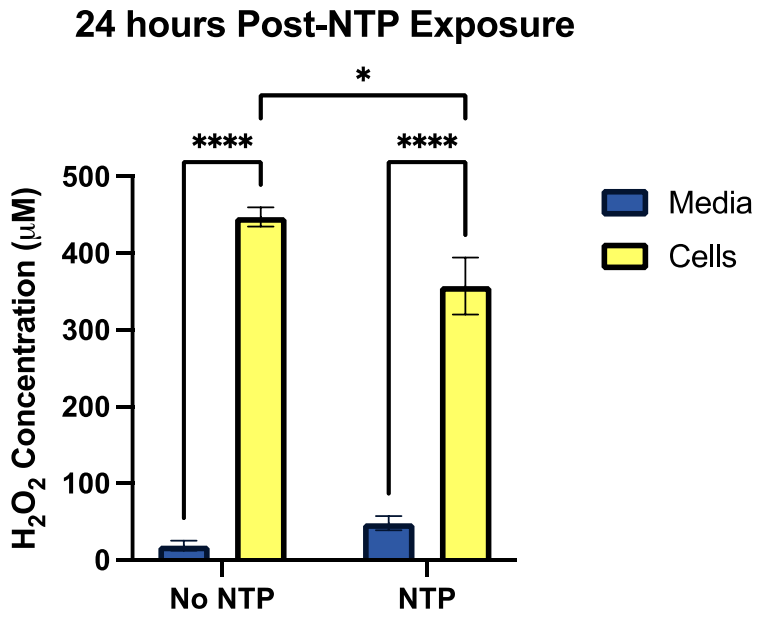

Figure S1. Hydrogen peroxide was measured in media and Vero cell media 24 h after NTP exposure. * $p = 0.0296$, **** $p < 0.0001$.

Over the course of 24 h following NTP exposure, hydrogen peroxide significantly increased in the media of live Vero cells, compared to media alone. In the absence of NTP, hydrogen peroxide concentrations in Vero cell media increased compared to media alone. This increase in concentration in the absence of NTP suggests that Vero cells may produce hydrogen peroxide as a byproduct of metabolism. In the presence of NTP exposure, hydrogen peroxide decreases in Vero cell media compared to unexposed Vero cell media. This decrease in concentration may be indicative of hydrogen peroxide reacting with other NTP RONS delivered to the media. Alternatively, this decrease could also be due to cellular antioxidant expression influenced by NTP that neutralizes hydrogen peroxide (Supplementary Figure S1). Lastly, in the presence and absence of live Vero cells, nitrite concentrations remain consistent 24 h following NTP exposure (Supplementary Figure S2).

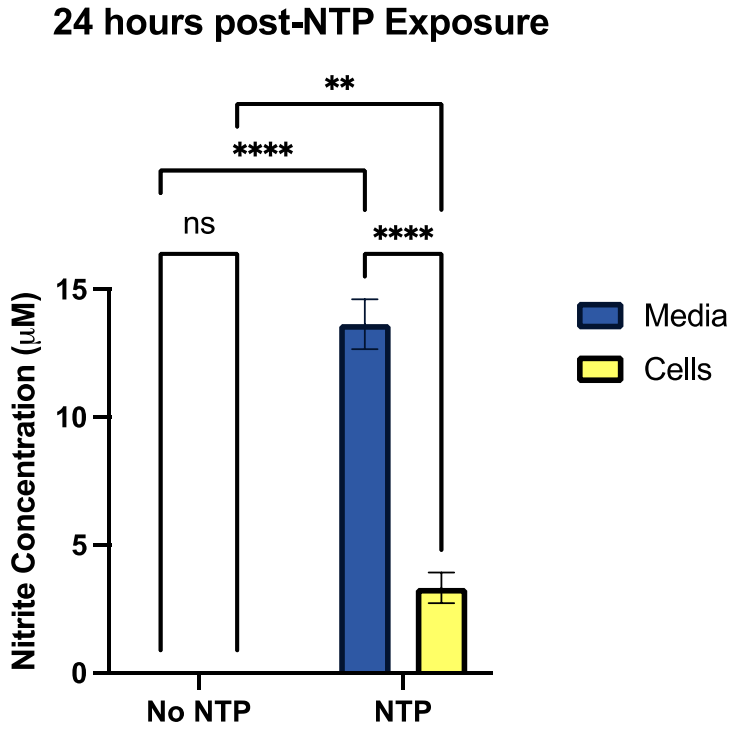

Figure S2. Nitrite was also measured in media alone and media in the presence of live Vero cells 24 h after NTP exposure. ** $p = 0.002$, **** $p < 0.0001$.