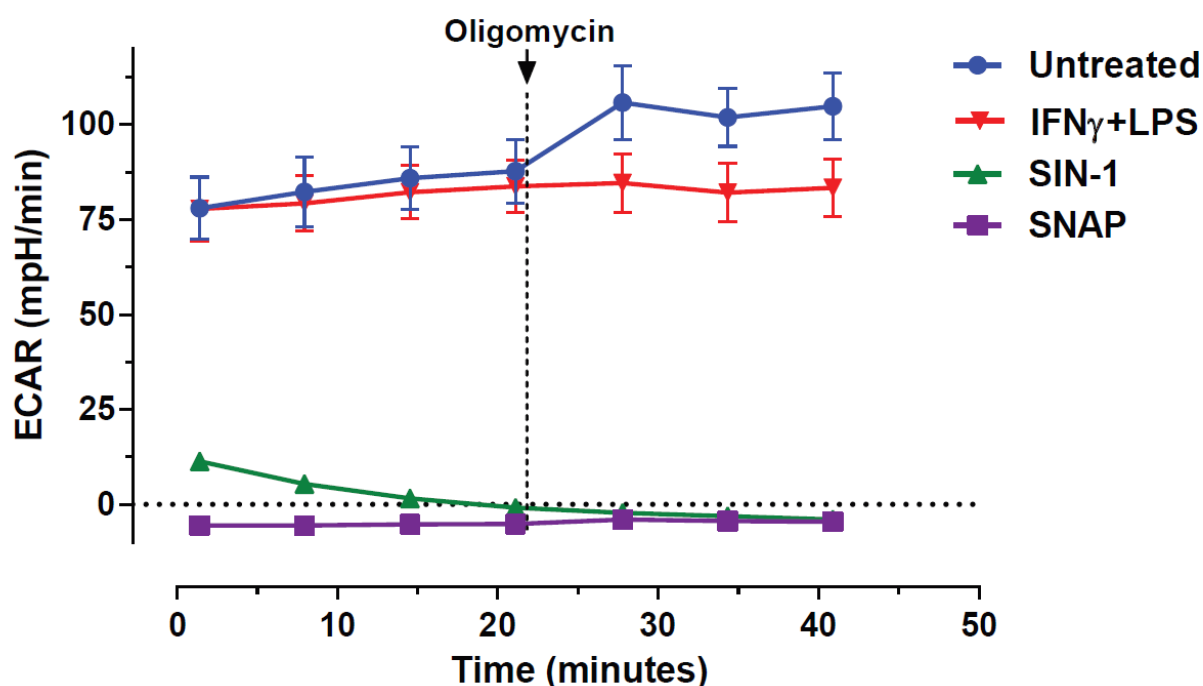
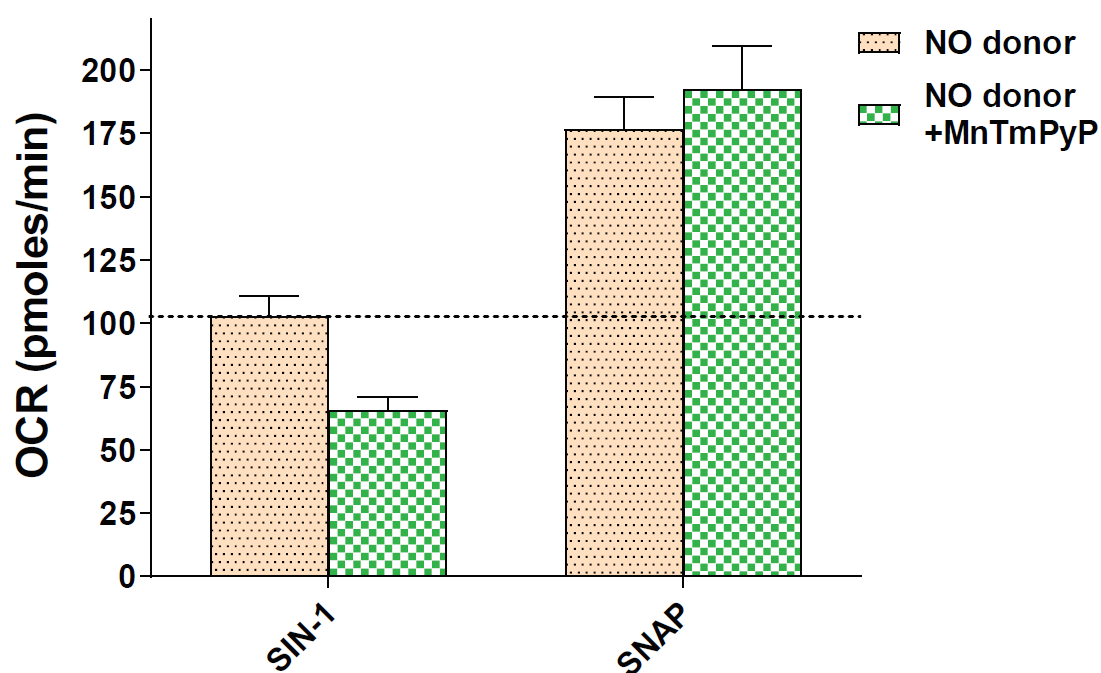




## Supplementary Material



**Figure S1.** NO $\bullet$  and O $2^{\bullet-}$  have no effect on the media acidification rates. Macrophages were activated for 24h with the IFN $\gamma$  + LPS (40ng/mL and 10ng/mL respectively) or not (untreated) and subjected to serial injections of oligomycin (2 $\mu$ M), FCCP (1 $\mu$ M) and antimycin A/rotenone (2/2 $\mu$ M), according to the mitostress protocol. A Seahorse XFe96 metabolic analyzer was used to record the changes in the media acidification rate (ECAR). The reactive species-donors SIN-1 (NO $\bullet$  and O $2^{\bullet-}$ ) or SNAP (NO $\bullet$ ) were added (3mM for either) prior to the assay in cell-free, media-only wells. Horizontal intermittent line indicate the minimum (zero) ECAR values. The corresponding ECAR values of the experiment in Figure 1 are shown, with data presented as mean  $\pm$  STD of at least three replicates per condition. Any ECAR values following the addition of FCCP are not shown, due to the interference of H $^{+}$  shuttled by the protonophore.



**Figure S2.**  $O_2^{\bullet-}$  scavenging lowers the cell-free oxygen consumption rates. The reactive species-donors SIN-1 ( $NO^{\bullet}$  and  $O_2^{\bullet-}$ ) or SNAP ( $NO^{\bullet}$ ) were added (3mM for either) to cell free-wells with or without the addition of the superoxide scavenger MnTmPyP (15  $\mu$ M). The Seahorse mitostress protocol was used to estimate the changes in the oxygen consumption rate (OCR). The lowest in value measurement following the addition of anti-mycin A/rotenone injection was used to calculate the differences in the oxygen consumption, following baseline subtraction. Horizontal intermittent line indicate the OCR levels of SIN-1. One representative experiment is shown, with data presented as mean  $\pm$  STD of at least three replicates per condition.