Alternative activation of anti-inflammatory macrophages in the lung by hyperbaric oxygen during *S.aureus* sepsis in mice

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Background

- ALI/ARDS are high-mortality complications of severe sepsis
- Sepsis related ARDS characterized by persistent elevation of pro-inflammatory cytokines in BAL with slow resolution
- Alveolar macrophages are principally responsible for cytokine production
- In general, tissue macrophages, can switch between a pro-inflammatory (M1) and anti-iflammatory (M2) phenotype thus capable of orchestrating the onset and resolution of tissue inflammation
- The effect of HBO_2 on alveolar macrophage (AM) sub-populations unknown

Hypotheses

We hypothesized:

1. HBO_2 induces the alternatively activated (M2) macrophage phenotype
2. Phenotype switch induces anti-inflammatory cytokine expression and attenuation of lung injury

We investigated these hypotheses in a murine sepsis model where we measured the effect of HBO_2 on AM phenotype and on key inflammatory and repair molecules in the lung

Survival Study

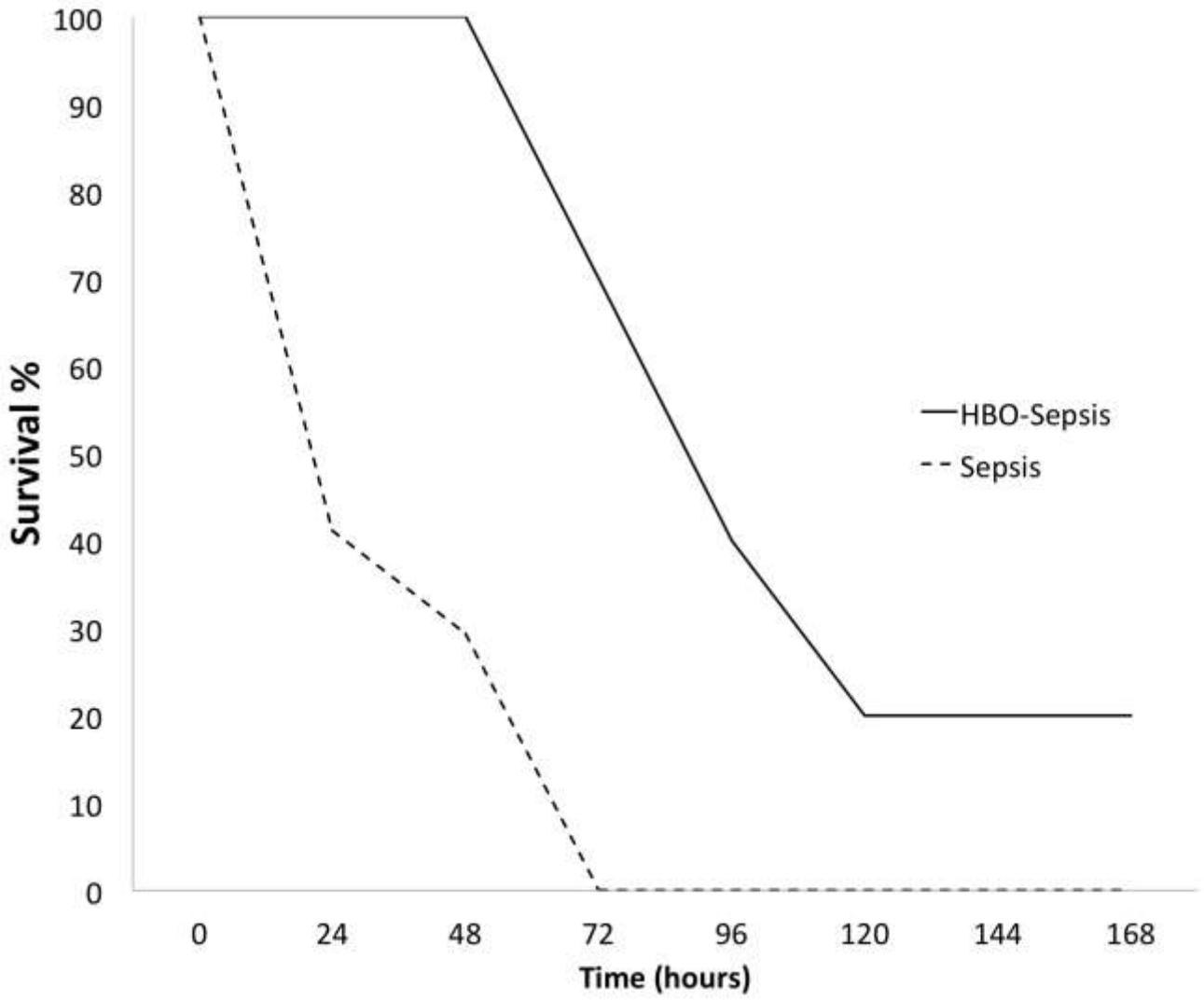
Mouse: BL-6 (Wild Type)

Sepsis: IP clot *S.Aureus* $1x10^8$ cfu

Antibiotic: Vancomycin 3mg/kg

Protocol: HBO_2 2.5 ata for 90mins x 3 treatments
(timed at 6hr, 24hr, 48hr post-inoculation)

HBO_2 improves *S.aureus* sepsis survival



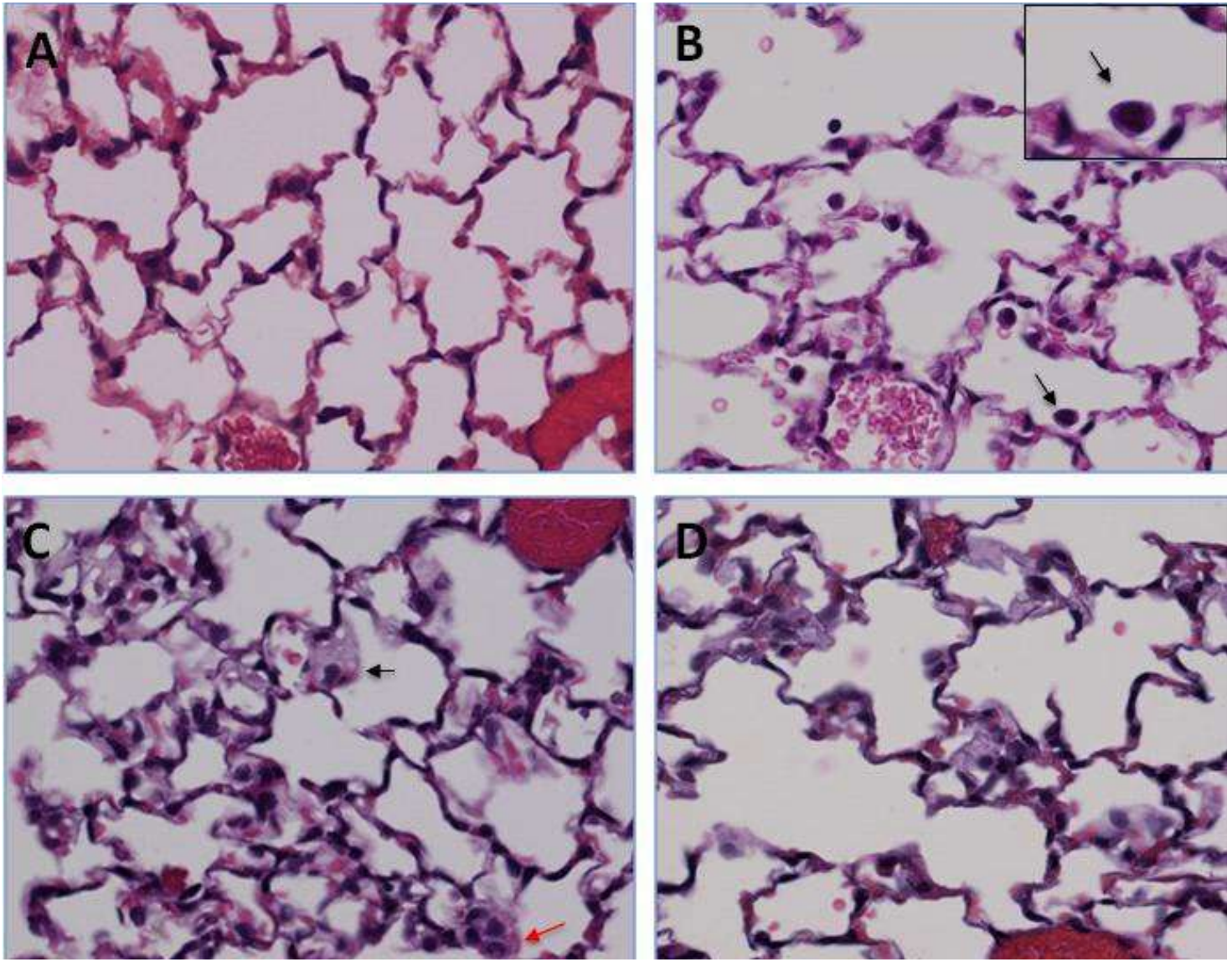
*P < 0.05 by Log rank test

Experimental Design*

Control	HBO_2 control	Sepsis	Sepsis + HBO_2
No interventions	2.5 ATA for 90 min at 0h, 24h, 48h	*S. aureus* $1x10^8$ CFU Vanc 6mg/kg 0.9%NaCl 1.0 ml sq	Sepsis + HBO_2 protocols

*n = 12 per group

HBO_2 attenuates lung inflammation at 24hrs



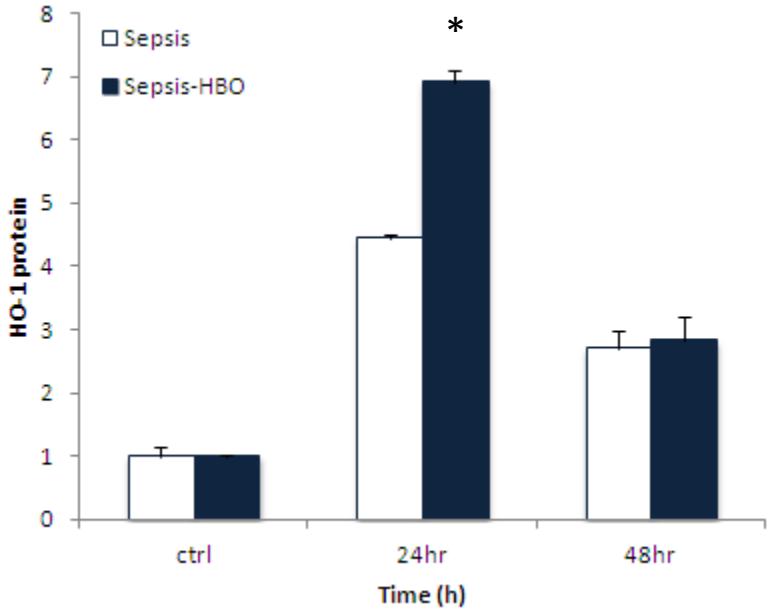
Control

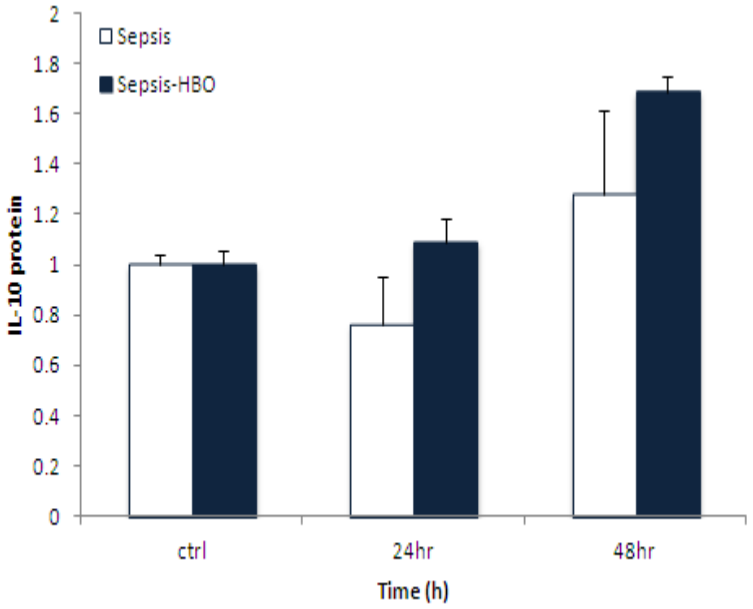
HBO_2

Sepsis

Sepsis + HBO_2

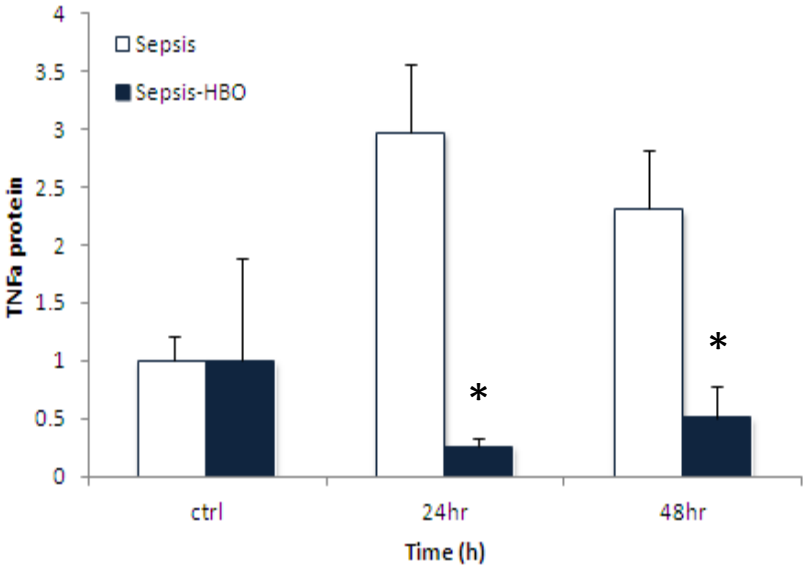
HBO_2 induces anti-oxidant and anti-inflammatory molecules

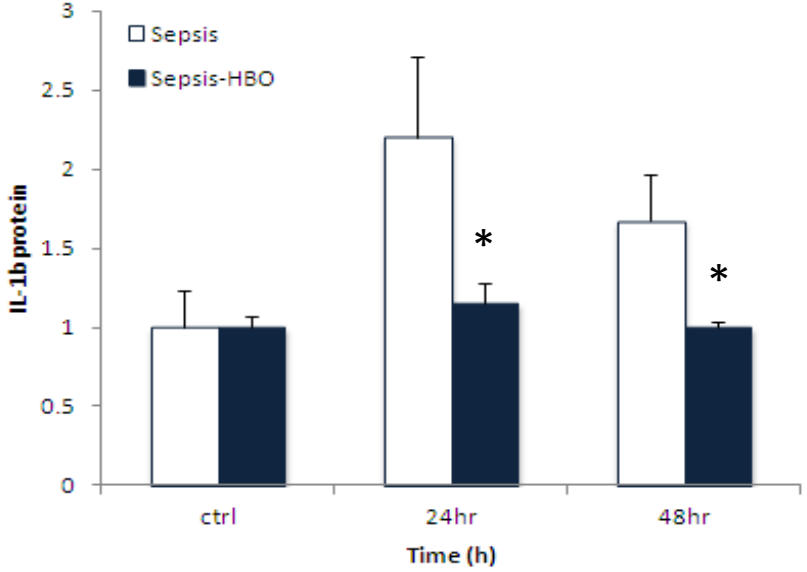




*(*P < 0.05 vs sepsis)*

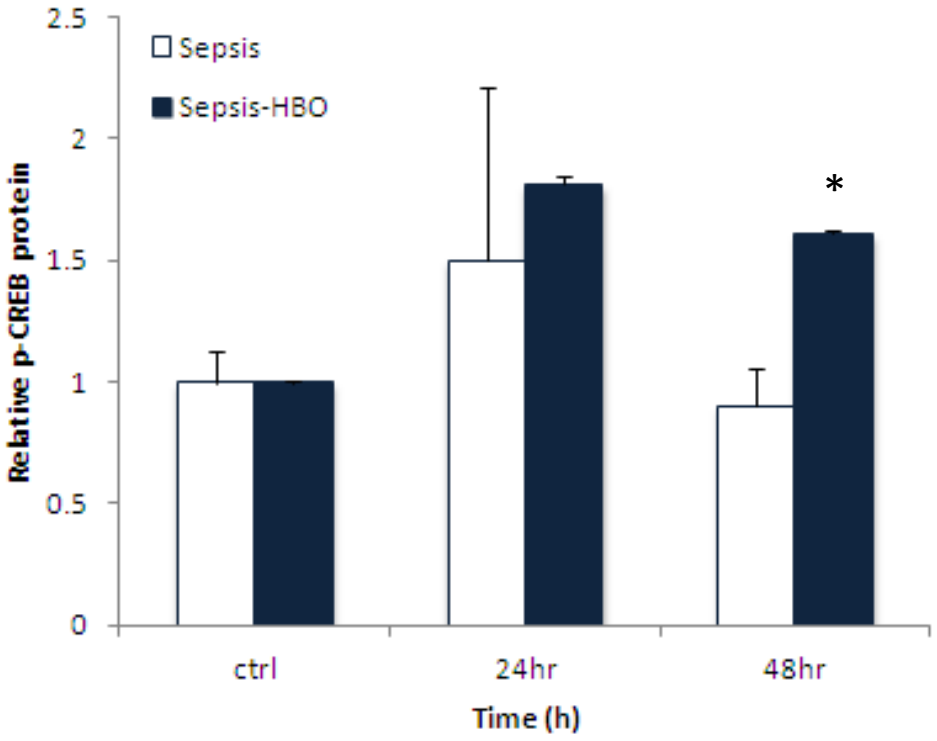
HBO_2 suppresses lung pro-inflammatory cytokines at 24hrs





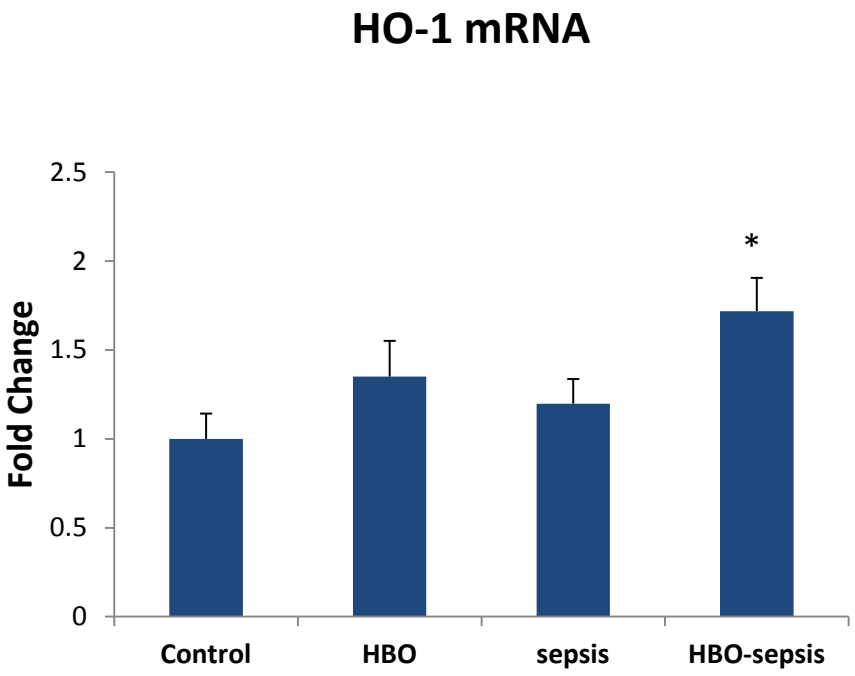
*(*P < 0.05 vs sepsis)*

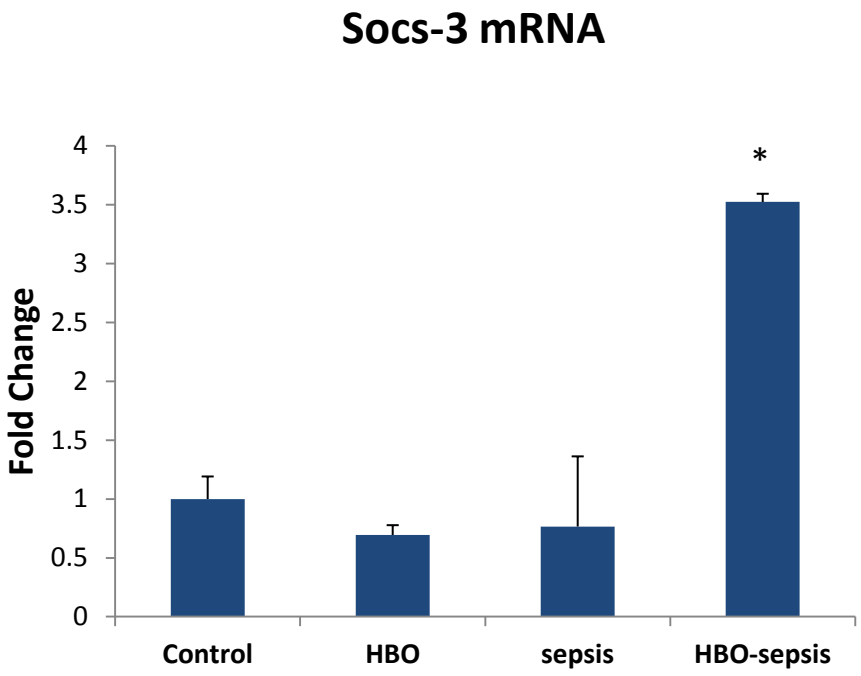
HBO_2 increases CREB phosphorylation by 48hrs



*(*P < 0.05 vs sepsis)*

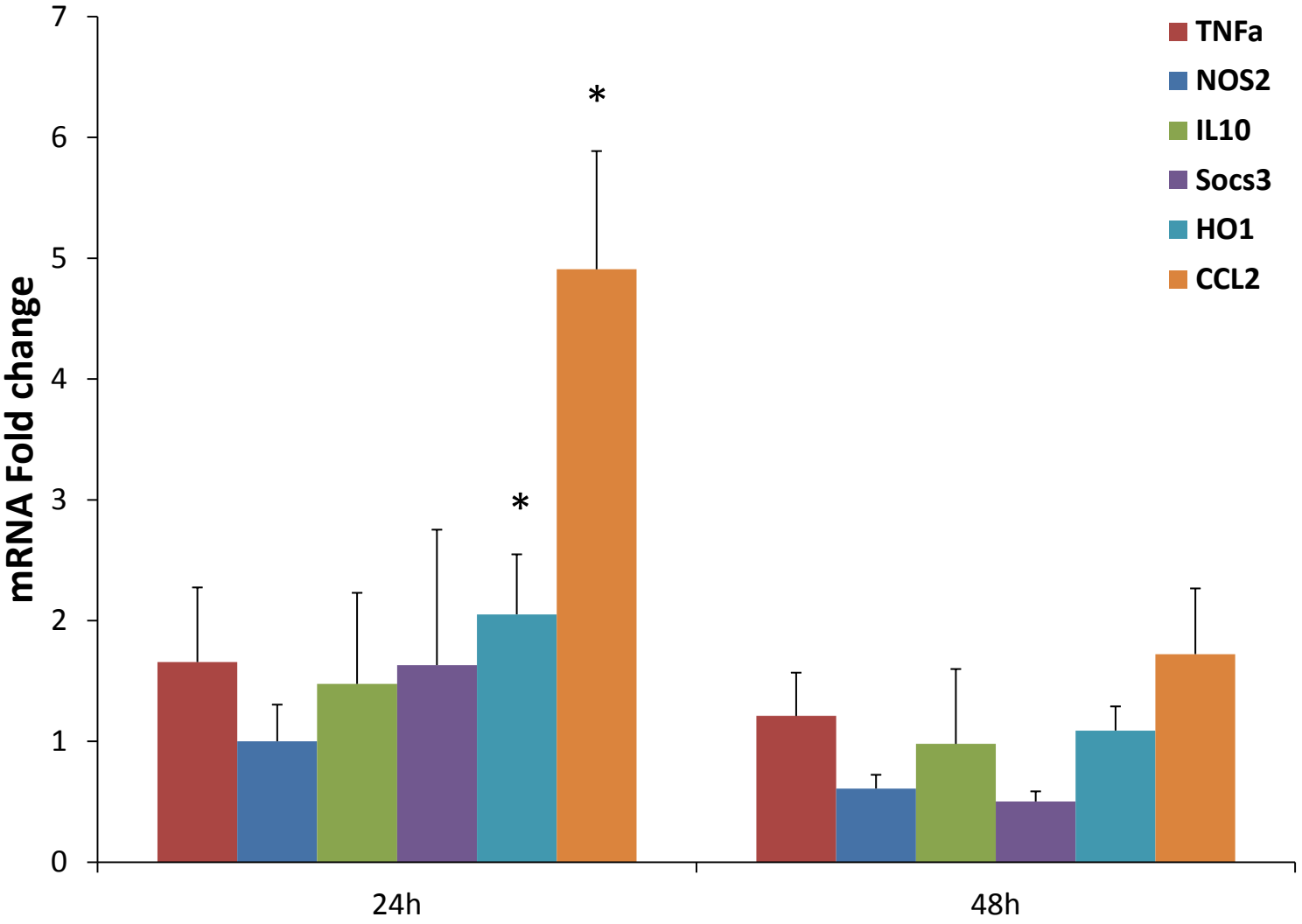
HBO_2 induces a lung-protective response to sepsis by 48hrs





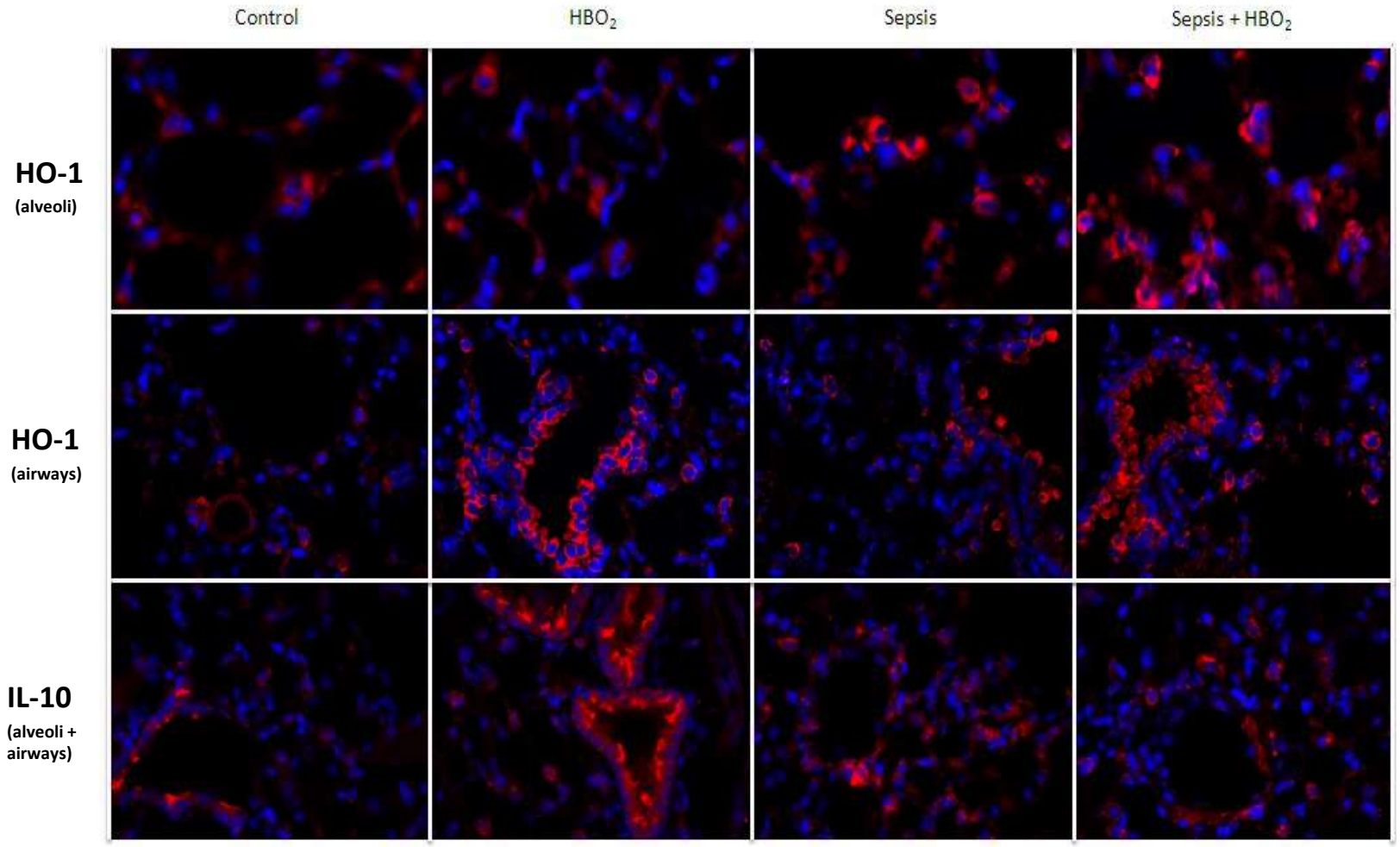
*(*P < 0.05 vs sepsis)*

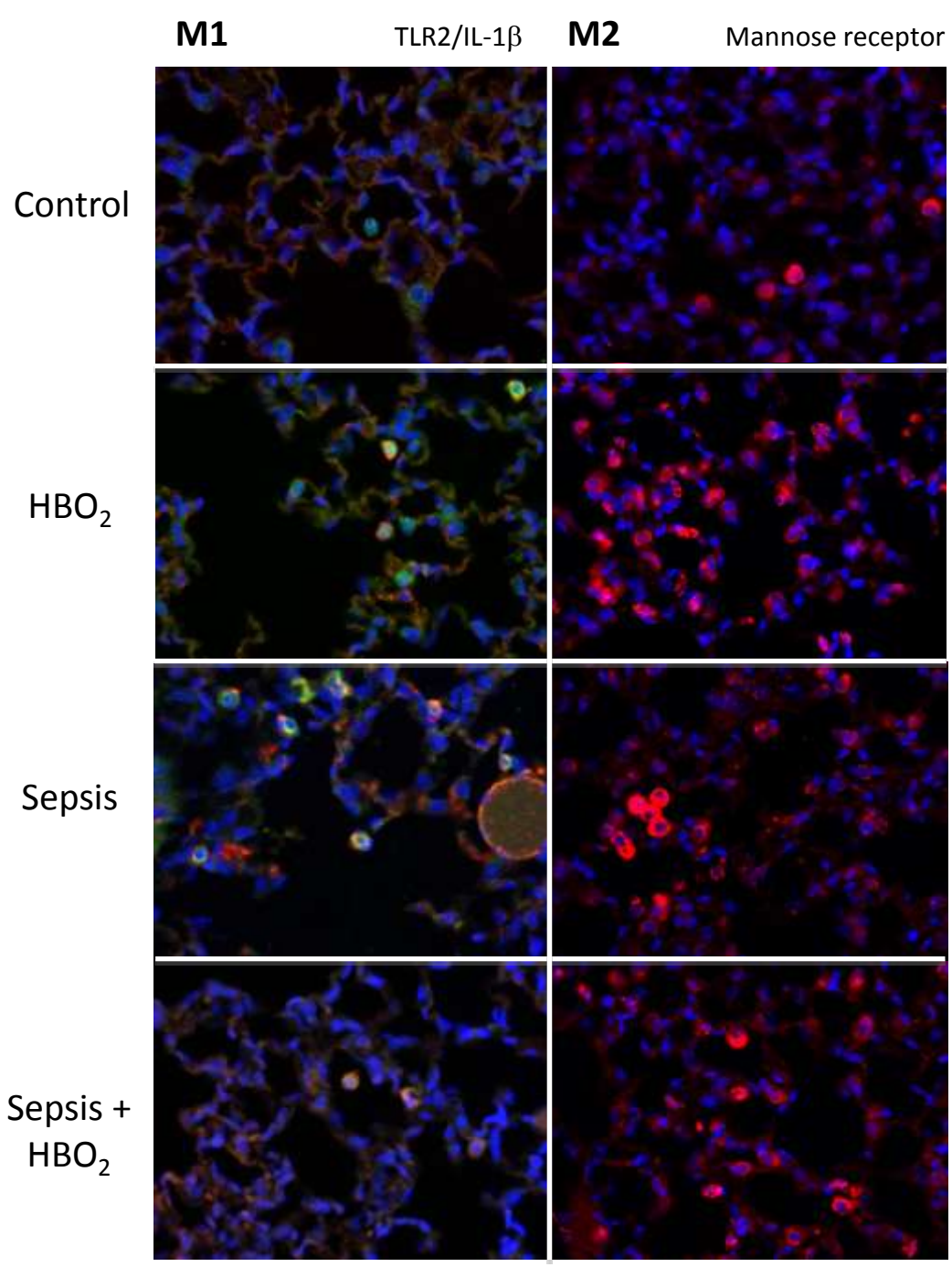
The effect of HBO_2 on inflammatory cytokines in healthy lung tissue

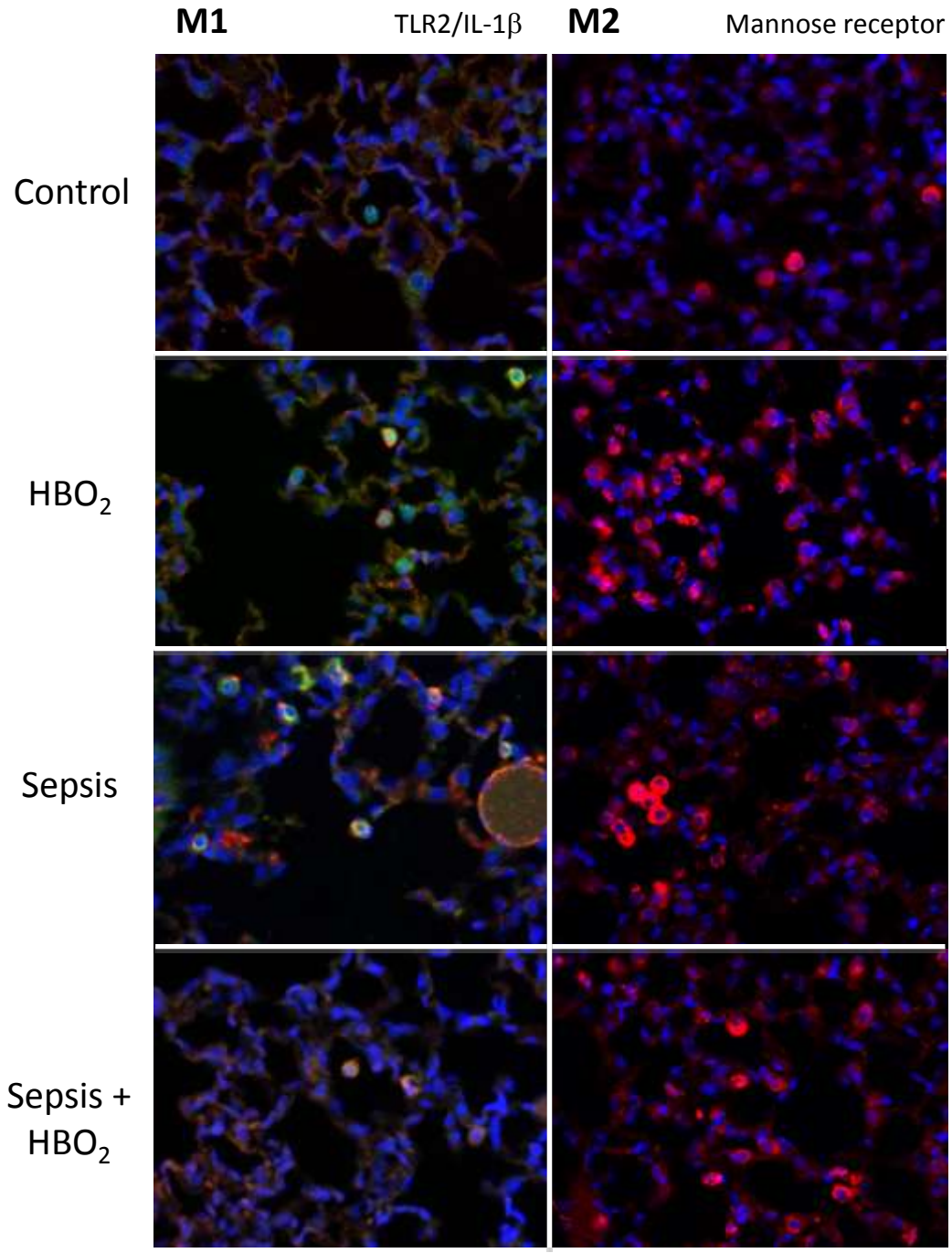


*(*P < 0.05 vs control)*

HBO_2 increases HO-1 and IL-10 expression in alveolar macrophages at 24h

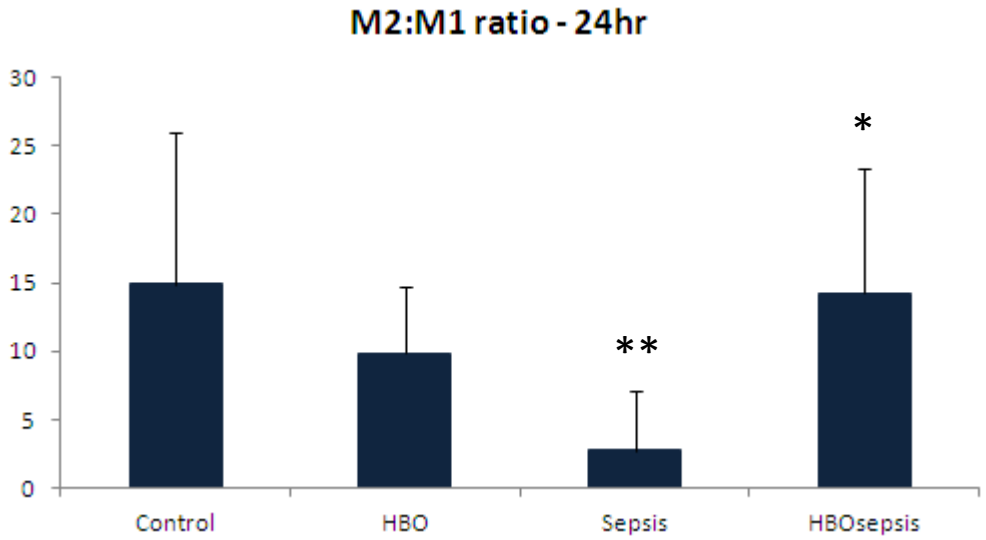


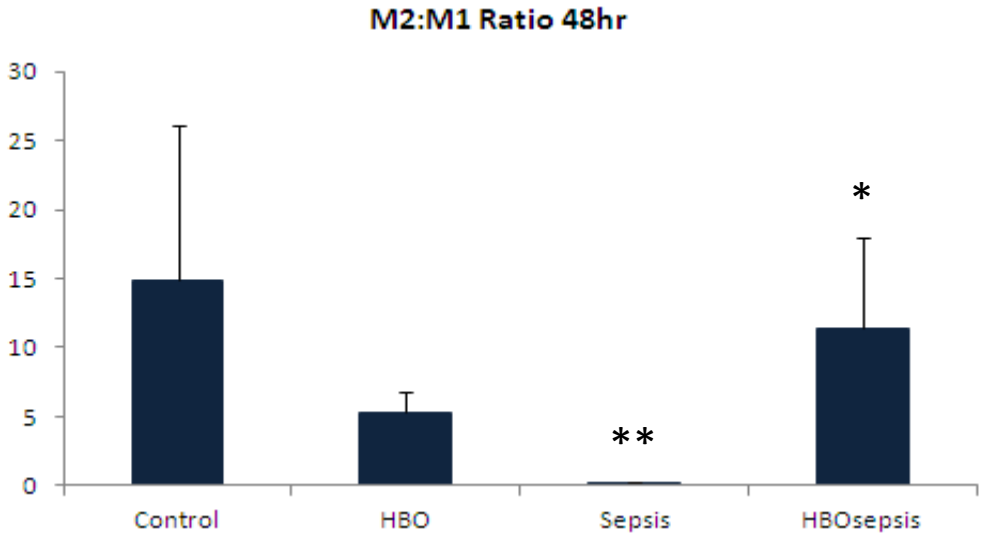

M1
TLR2/IL-1β
M2
Mannose receptor
Control
HBO2
Sepsis
Sepsis + HBO2



HBO_2 switches AM phenotype from M1 to M2 during sepsis

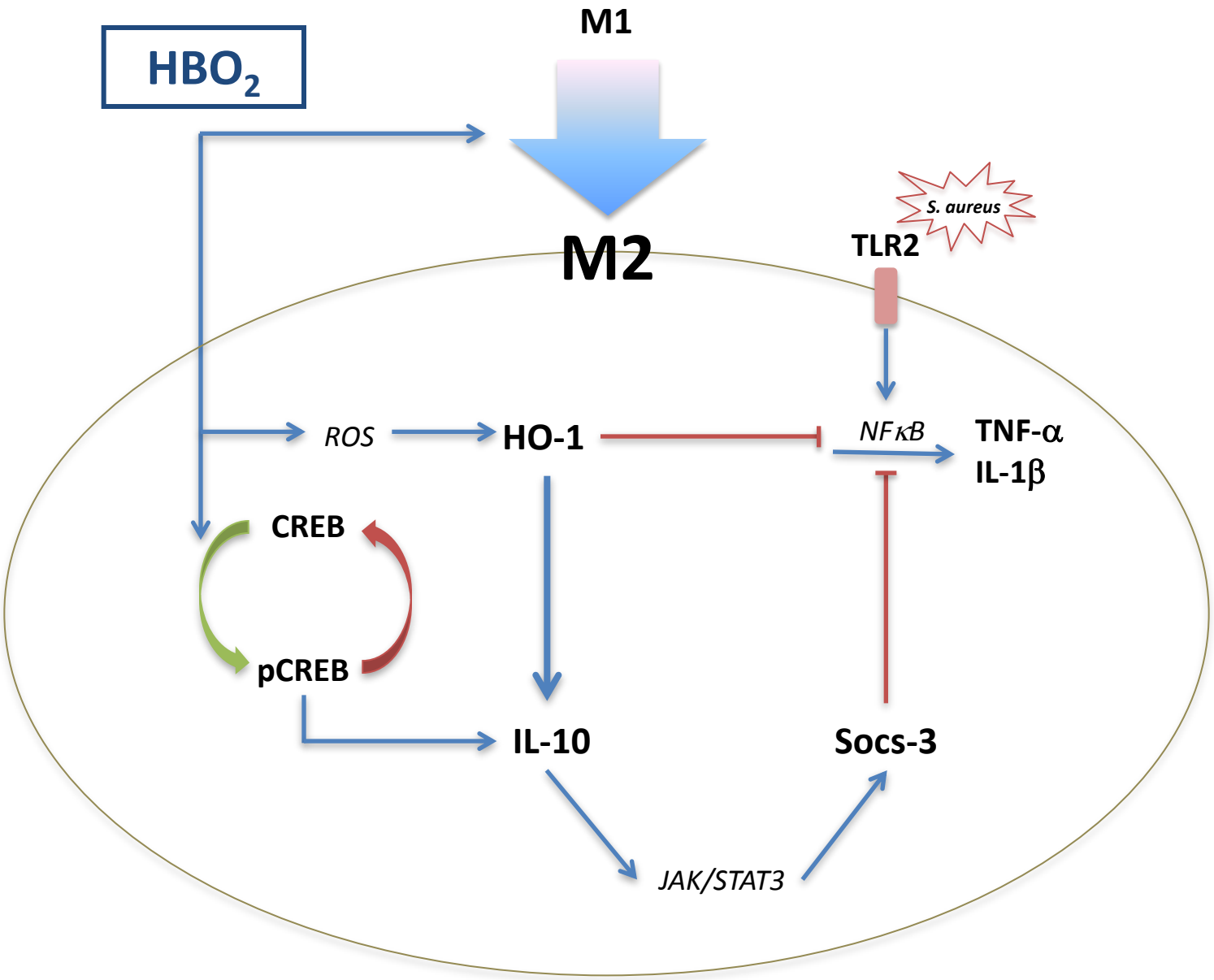
HBO_2 induces M2 macrophage expression in sepsis





*(*P < 0.05 vs sepsis; **P < 0.05 vs control)*

Putative Mechanism



Conclusions

- HBO_2 attenuates lung inflammation and improves survival in *S.aureus* sepsis
- HBO_2 suppresses early-phase pro-inflammatory and up-regulates the anti-inflammatory response to sepsis in the mouse lung
- HBO_2-mediated alternative activation of alveolar macrophages may account for an anti-inflammatory effect of HBO_2 in the lung during sepsis.