

A single dose of eHSP72 administration attenuates sepsis severity in mice

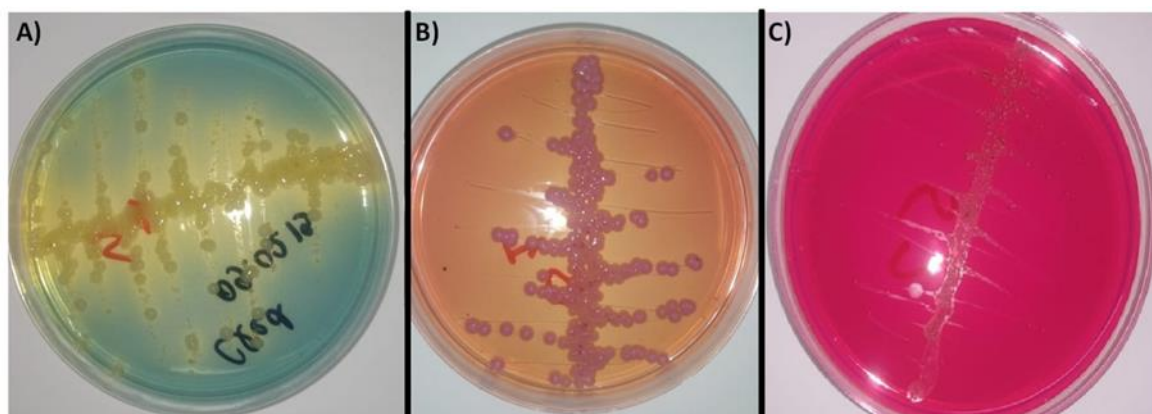
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Supplementary material 1.

Supplementary methods:



Supplementary Figure 1. Representative images from bacterial analyses of faecal solution administered in mice for sepsis induction. Faecal solution was prepared in sterile NaCl 0.9% solution (100 mg/ml). Bacterial colonies were analysed by two independent evaluators. (A) In the Agar Cled medium (INTERLAB) (A) were identified colonies of *Escherichia coli* and *Staphylococcus aureus*. Thus, the CFU count were performed in (B) Agar MacConkey (HIMEDIA) and (C) Agar Sal mannitol IO053 (IONLAB) by incubation at 35 °C (± 2) by 24 h. We found 216 ± 0.707 colonies of *E. coli* and 494 ± 9.192 of *S. aureus*. Since the sample volume used in this analysis was 50 μ L, representing five milligrams of faeces and was administered at the dose of 1 mg/g per animal, we estimated that 43 CFUs/g of *E. coli* and 99 CFUs/g of *S. aureus* were i.p. administered for sepsis induction.

Murine Sepsis Score (MSS) (SHRUM et al., 2014)

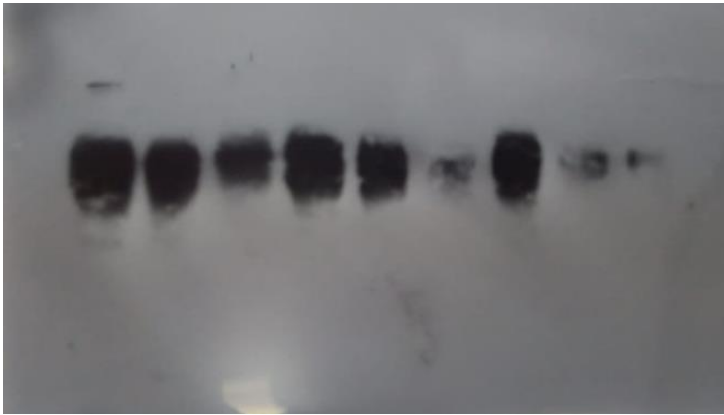
Variable	Score and description
Appearance	0- Coat is smooth 1- Patches of hair piloerected 2- Majority of back is piloerected 3- Piloerection may or may not be present, mouse appears “puffy” 4- Piloerection may or may not be present, mouse appears emaciated
Level of consciousness	0- Mouse is active 1- Mouse is active but avoids standing upright 2- Mouse activity is noticeably slowed. The mouse is still ambulant. 3- Activity is impaired. Mouse only moves when provoked, movements have a tremor 4- Activity severely impaired. Mouse remains stationary when provoked, with possible tremor
Activity	0- Normal amount of activity. Mouse is any of: eating, drinking, climbing, running, fighting 1- Slightly suppressed activity. Mouse is moving around bottom of cage 2- Suppressed activity. Mouse is stationary with occasional investigative movements 3- No activity. Mouse is stationary 4- No activity. Mouse experiencing tremors, particularly in the hind legs
Response to stimulus	0- Mouse responds immediately to auditory stimulus or touch 1- Slow or no response to auditory stimulus; strong response to touch (moves to escape) 2- No response to auditory stimulus; moderate response to touch (moves a few steps) 3- No response to auditory stimulus; mild response to touch (no locomotion) 4- No response to auditory stimulus. Little or no response to touch. Cannot right itself if pushed over
Eyes	0- Open 1- Eyes not fully open, possibly with secretions 2- Eyes at least half closed, possibly with secretions 3- Eyes half closed or more, possibly with secretions 4- Eyes closed or milky
Respiration rate	0- Normal, rapid mouse respiration 1- Slightly decreased respiration (rate not quantifiable by eye) 2- Moderately reduced respiration (rate at the upper range of quantifying by eye) 3- Severely reduced respiration (rate easily countable by eye, 0.5 s between breaths) 4- Extremely reduced respiration (>1 s between breaths)
Respiration quality	0- Normal 1- Brief periods of laboured breathing 2- Laboured, no gasping 3- Laboured with intermittent gasps 4- Gasping

Supplementary data:

Original immunoblots images supplementary information of figure 6d (HSP70 / β -actin expression in lung of mice).

Bands represents HSP70 expression in the lung of mice.

The sequence of the Blots is Control; Sepsis and Sepsis+eHSP72 (repeatedly)



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