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Supplementary Information for

Beneficial effects of citrulline enteral administration on sepsis induced T-cell mitochondrial dysfunction

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Figures S1 to S4

SI FIGURE LEGENDS

Fig. S1.

Lung Injury Scoring System

Fig. S2.

Gating strategy for the identification of mouse cell subsets (regulatory T-cells, neutrophils, monocytes, M-MDSC and G-MDSC) in whole blood. Doublets and dead cells (Dapi^{pos}) were excluded before backgating of CD19/CD335/Ter119/CD41^{pos} cells (Lin). Regulatory T-cells were gated on Lin^{neg}CD3^{pos} cells as CD25^{pos} and CD127^{neg}. Neutrophils were defined as Lin^{neg}Ly-6G^{pos}. Monocytes as Lin^{neg}Ly-6G^{neg}/CD11b^{pos}/CD11c^{neg}. MDSC subsets were defined on Lin^{neg} and CD11b^{pos} after elimination of neutrophils and monocytes and based on Ly-6C and Ly-6G expression.

Fig. S3.

(A) Flow cytometry analysis of spleen T-cells cell cycle shows that CLP does not induce modification in cell cycle to compare with sham mice (n=14).

(B) Expression of *CD247/CD3z* was determined by Q-PCR on purified spleen T cells. Results are presented as the ratio between *CD3z* and *CD3e* expression in order to normalize the data for the CD3_{pos} cell frequency (Sham n=6; CLP n=6; Placebo n=6; Arginine n=6; Citrulline n=6).

(C) Nitric Oxide concentrations were measured using the Griess reaction in the culture supernatants of peripheral PBMC stimulated with anti-CD3 and anti-CD28 antibodies (Sham n=12; CLP n=12; Placebo n=9; Arginine n=9; Citrulline n=9)

* p<.05

Fig. S4.

(A) Extracellular acidification rate (ECAR) was measured in peripheral blood CD3^{pos} T-cells (Sham n=5; CLP n=5; Placebo n=4; Arginine n=4 and Citrulline n=4).

(B) ECAR analysis of spleen T-cells (Sham n=7; CLP n=7; Placebo n=7; Arginine n=7 and Citrulline n=7).

(C) Western Blot analysis of OXPHOS-related proteins in purified spleen T-cells (Placebo n=8, Arginine n=8, and Citrulline n=8).

(D) Kaplan-Meier survival curves. Survival proportions were respectively of 85.5% (100/117), 84.7% (100/118), and 91.5% (108/118) for placebo arginine and citrulline treated mice.

* p<.05

Figure S1

Score per field			
Parameter	0	1	2
1. Neutrophils in the alveolar space	none	1-5	>5
2. Neutrophils in the interstitial space	none	1-5	>5
3. Hyaline membranes	none	1	>1
4. Proteinaceous debris filling the airspaces	none	1	>1
5. Alveolar septal thickening	<2x	2x-4x	>4x

$$\text{Score} = [(20 \times A) + (14 \times B) + (7 \times C) + (7 \times D) + (2 \times E)] / (\text{number of fields} \times 100)$$

Figure S2

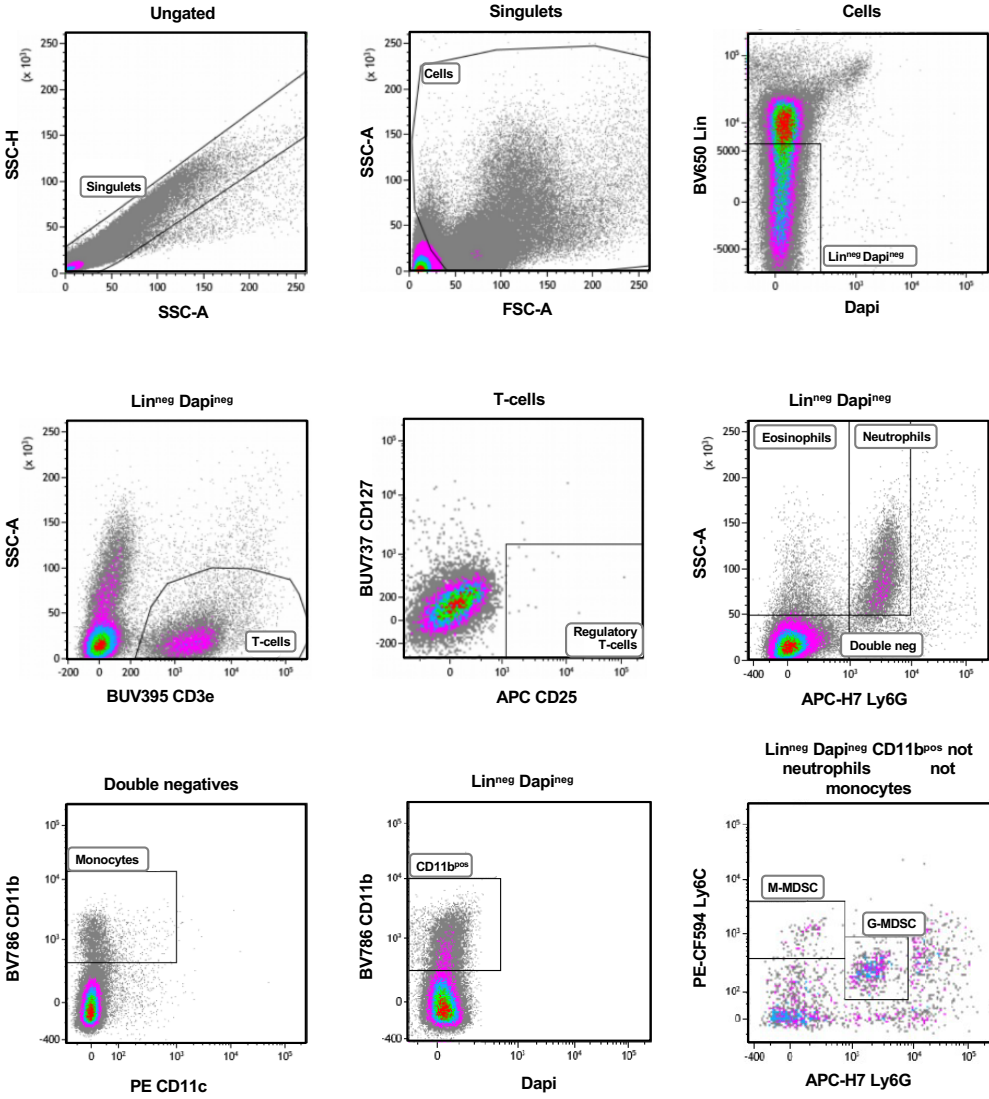
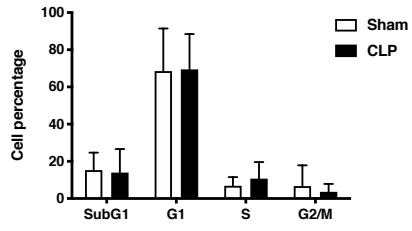
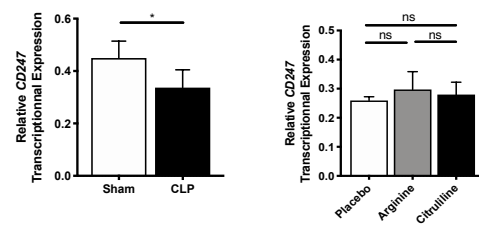


Figure S3

A



B



C

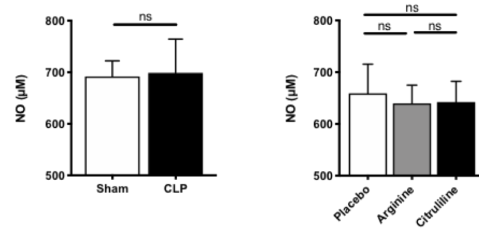
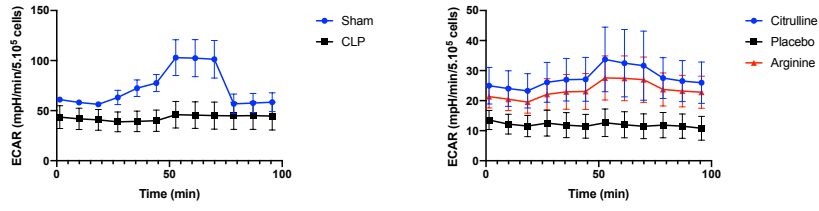
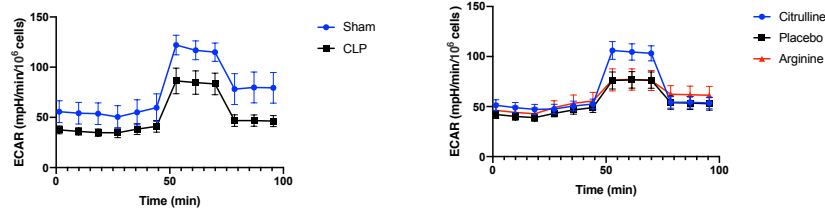


Figure S4

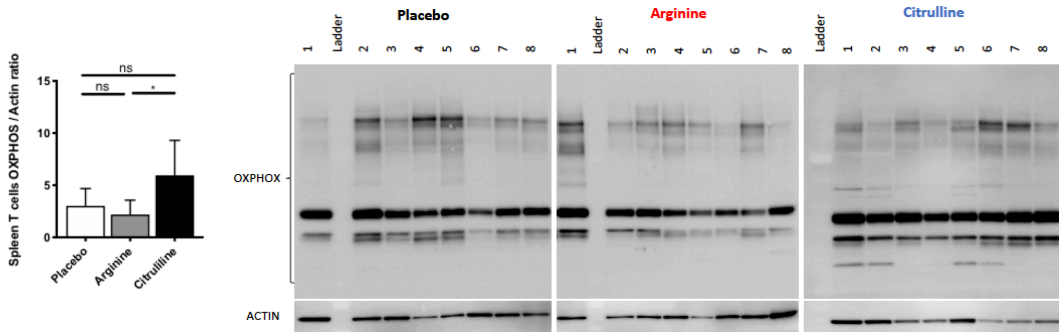
A



B



C



D

