SUPPLEMENTARY INFORMATION

Supplementary Table 1. Demographic characteristics of emergency department patients with sepsis.

Supplementary Table 2. Demographic characteristics of emergency department patients without sepsis.

<u>**Supplementary Figure Legends**</u>

Supplementary Figure 1. Autocrine purinergic signaling is required for antigen recognition.

(A) Jurkat cells were treated or not (control) for 20 min with CCCP (10 µM) and stimulated with anti-CD3/CD28 antibody-coated beads. Immune synapse formation was assessed 5 min after addition of beads (63x oil objective, NA 1.4; scale bar, 5 µm). (B) Jurkat cells were treated or not with suramin (100 µM) for 10 min, stimulated with anti-CD3/CD28 antibody-coated beads for another 10 min and cell/bead interactions were analyzed by flow cytometry. Cells associated with beads were identified by their characteristic increase in side scattering properties. (C) Jurkat cells were treated with suramin (100 µM) for 10 min, stimulated with anti-CD3/CD28 antibody-coated beads for another 10 min, and mitochondrial ROS production was assessed using DHR123 and flow cytometry. Cells undertaking ROS production were gated as shown (DHR$^+$).

Supplementary Figure 2. Mitochondrial dysfunction correlates with impaired Ca^{2+} signaling and sepsis severity and is not due to a reduction in mitochondrial mass or P2X receptor expression.

(A) Leukocytes of patients with sepsis (n=9) or patients without sepsis (n=9) or healthy controls (n=7) were stained with MitoTracker Green AM and analyzed by flow cytometry. (B) T cells

were purified from the blood of sepsis patients (n=12) or healthy control subjects (n=12) and

mRNA levels of P2X1, P2X4 and P2X7 receptors were assessed by qPCR; ***$p<0.001$, t test.

(C) Leukocytes of healthy subjects (n=12), non-septic patients (n=7) or septic patients (n=10)

were stained with DHR123 or Fluo-4 and analyzed by flow cytometry. Ca^{2+} levels in $CD4^+$ T

cells were assessed before and 3 min after stimulation by TCR/CD28 cross-linking. The

associations between mitochondrial ROS production and basal and stimulated Ca^{2+} levels were

analyzed with Pearson's correlation analysis. (D) Correlation between mitochondrial ROS

production and the Sequential Organ Failure Assessment (SOFA) score, a clinical measure of

sepsis severity (determined on day 1 of sepsis diagnosis) was assessed with Pearson's correlation

analysis. $CD4^+$ T cells were identified by forward/side scattering and CD4 staining; n.s., non-

significant; MFI, mean fluorescence intensity; r, Pearson's correlation coefficient.

Supplementary Figure 3. Mitochondrial ROS formation, mitochondrial membrane

potential and Ca^{2+} signaling are not affected by age or gender.

Leukocytes of healthy subjects, non-septic or septic emergency department patients were stained

with DHR123, TMRE or Fluo-4 and analyzed by flow cytometry. Correlations between age and

(A) mitochondrial ROS production, (B) mitochondrial membrane potential $\Delta\Psi m$, (C) Ca^{2+} levels

3 min after stimulation by TCR/CD28 cross-linking or correlations between sex and (D)

mitochondrial ROS production, (E) membrane potential and (F) stimulated Ca^{2+} levels were

analyzed with Pearson's correlation analysis separately for the sepsis group and the non-septic

control group. 60-70% of the subjects in each group were female. $CD4^+$ T cells were identified

by forward/side scattering and CD4 staining; n.s., non-significant; MFI, mean fluorescence

intensity; r, Pearson's correlation coefficient.

Supplementary Figure 4. Different stages of T cell activity depend on different levels of autocrine purinergic signaling.

T cells of patients with sepsis lack the autocrine purinergic signaling loop that maintains basal cytosolic Ca^{2+} levels and mitochondrial activity, resulting in a state of dormancy that impairs cellular vigilance. Under normal conditions, resting cells maintain vigilance through autocrine purinergic signaling that involves P2X1 receptors. In this state, mitochondria provide the ATP that stimulates P2X1 receptors and maintains basal Ca^{2+} homeostasis, allowing cells to actively recognize danger signals in their extracellular environment. Vigilant T cells can rapidly respond to TCR/CD28 stimulation by upregulating the autocrine purinergic signaling mechanisms that trigger mitochondrial activity, increase ATP release, and fuel Ca^{2+} influx by the activation of P2X1, P2X4 and P2X7 receptors, resulting in sustained cytosolic Ca^{2+} levels that are necessary for full-fletched functional T cell responses such as IL-2 production and proliferation. In sepsis, mitochondrial dysfunction disrupts the basal purinergic signaling loop that maintains T cell vigilance, resulting in T cell suppression and immune dysfunction.

<u>Supplementary Video Legends</u>

Supplementary Video 1. Suramin depletes mitochondrial and cytosolic Ca^{2+} signaling (related to Figure 1B).

Jurkat cells co-expressing the mitochondrial and cytosolic Ca^{2+} biosensors mito-CAR-GECO1 and G-GECO1.1 were treated or not (control) with 100 µM suramin for 20 min. Mitochondrial (red) and cytosolic (green) Ca^{2+} levels before and after stimulation via TCR crosslinking (at t=15 s) were imaged by fluorescence microscopy. Image pairs were merged with ImageJ software. Objective: 63x oil (NA 1.4); frame rate: 60 frames min^{-1}; scale bar, 5 µm.

Supplementary Video 2. Unstimulated T cells release ATP into the extracellular space (related to Figure 2A).

Jurkat T cells were stained with the membrane-targeting ATP probe 2-2Zn(II) and fluorescence and bright field time-lapse images were acquired. At t= 5 min and 42 s, ATP (100 µM) was added as a positive control. Objective: 100x oil (NA 1.30); frame rate: 30 frames min^{-1}.

Supplementary Video 3. Constitutive versus stimulation-induced ATP release (related to Figure 2A).

Jurkat cells were loaded with the membrane-bound ATP probe 2-2Zn(II), then anti-CD3/CD28 antibody-coated beads were added (small circular grey objects), resulting in the stimulation of some cells. Fluorescence time-lapse imaging was started immediately after addition of beads. At t=13 min 20 s, ATP (100 µM) was added as a positive control. At t= 15 min 0 s, apyrase (10 U/ml) were added as a negative control. Objective: 63x oil (NA 1.4); frame rate: 12 frames min^{-1}; scale bar, 10 µm.

Supplementary Video 4. Blocking mitochondrial function impairs T cell vigilance (related to Figure 2 and Supplementary Figure 1)

Jurkat cells were treated or not (control) with CCCP (10 µM) for 20 min and stimulated with anti-CD3/CD28 antibody-coated beads and the interactions of cells with beads were recorded. Cells treated with antibody-free beads served as negative controls. Objective: 63x oil (NA 1.4); 60 frames min^{-1}; scale bar, 5 µm.

Supplementary Video 5. Inhibition of autocrine purinergic signaling blocks mitochondrial activation and immune synapse formation (related to Figure 2 and Supplementary Figure 1).

Jurkat cells expressing the mitochondrial Ca^{2+} biosensor mito-CAR-GECO1 were treated or not (control) for 20 min with suramin (100 µM) or CCCP (10 µM). Then anti-CD3/CD28 antibody-coated beads were added and mitochondrial activation and bead interactions were recorded. Objective: 63x oil (NA 1.4); frame rate: 60 frames min^{-1}; scale bar, 5 µm.

**Supplementary Video 6. Inhibition of autocrine purinergic signaling depletes mitochondrial activity of resting cells (related to Figure 3 C and D).**

Jurkat cells were incubated or not (control) with suramin (100 µM) or CCCP (10 µM) for 20 min and mitochondrial activity was monitored with TMRE (100 nM) added at t=6 s. Objective: 100x oil (NA 1.30); frame rate: 30 frames min^{-1}; scale bar, 5 µm.
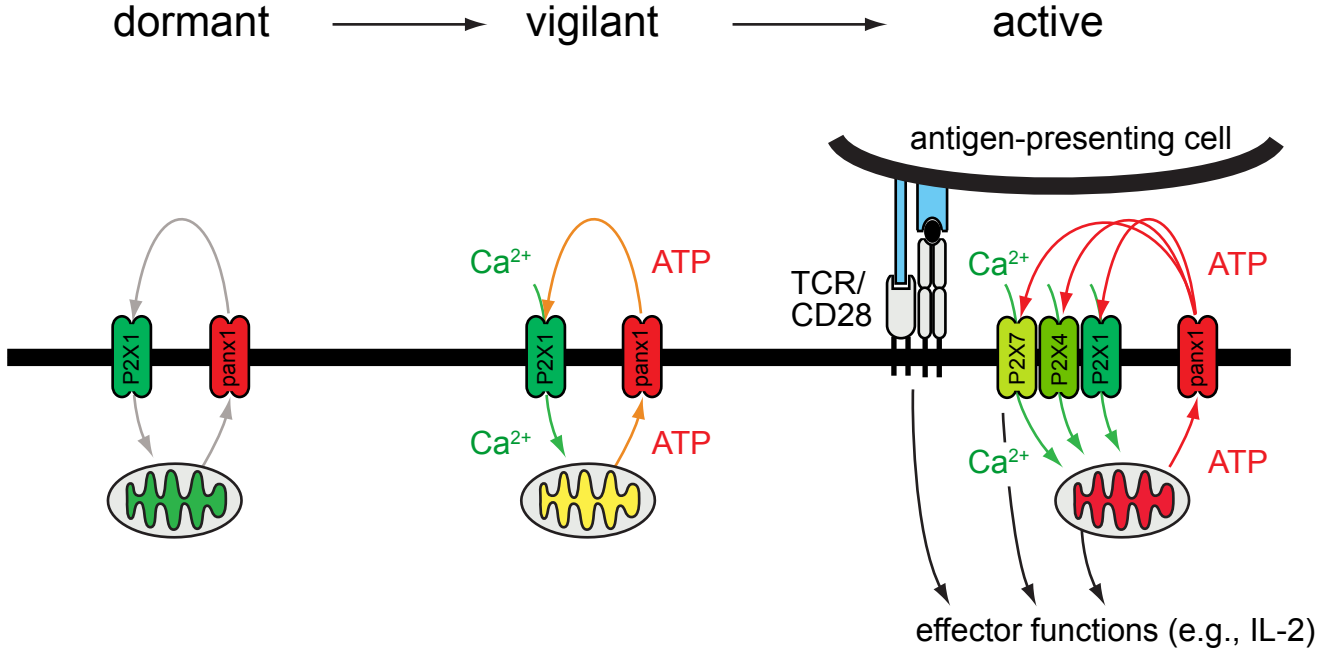
A

B

C

D
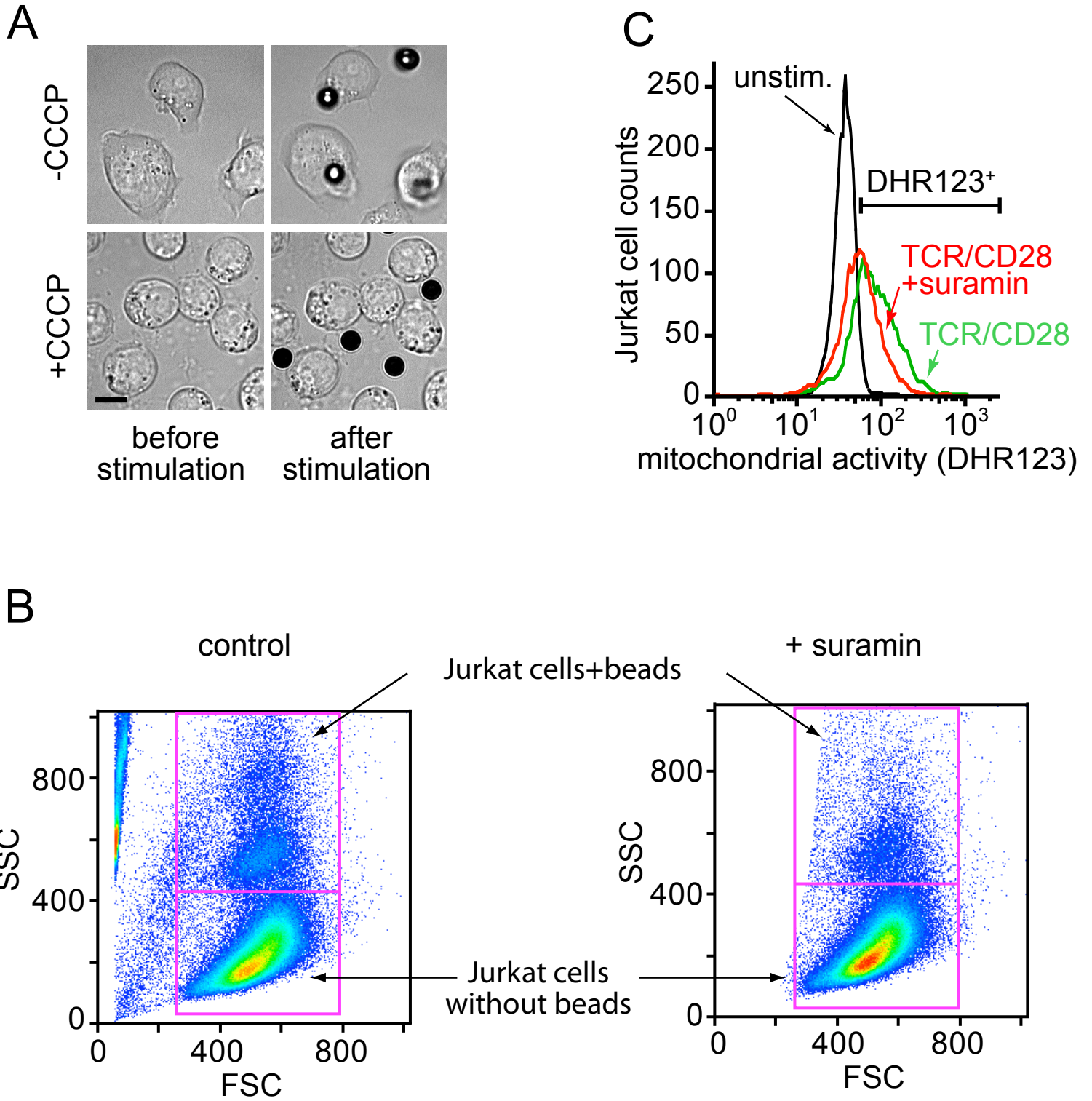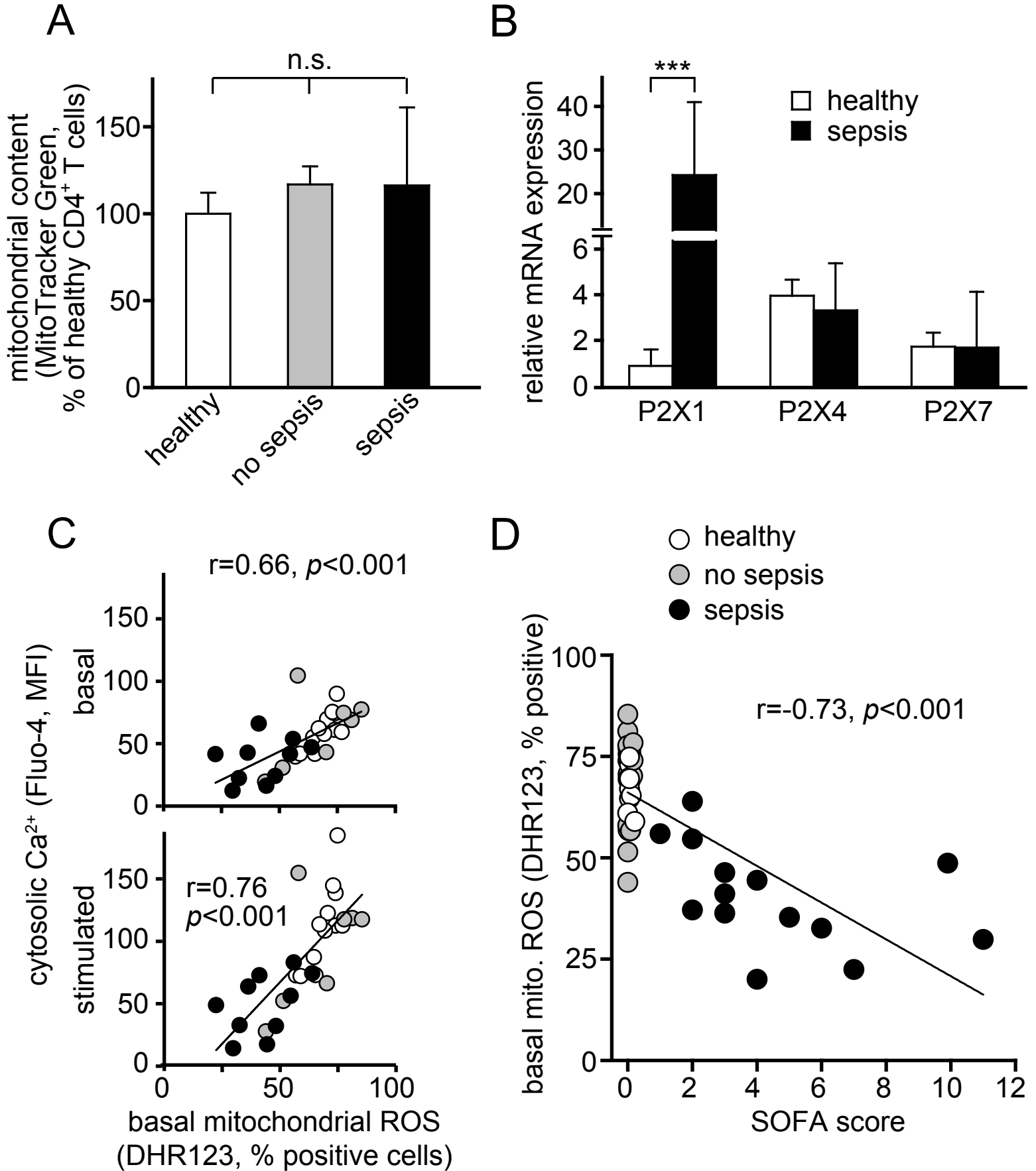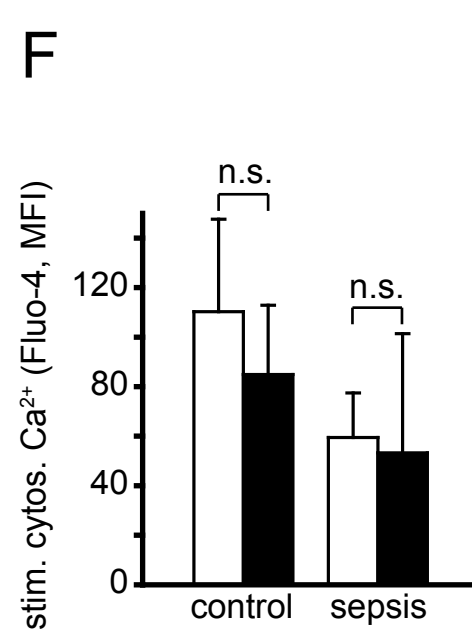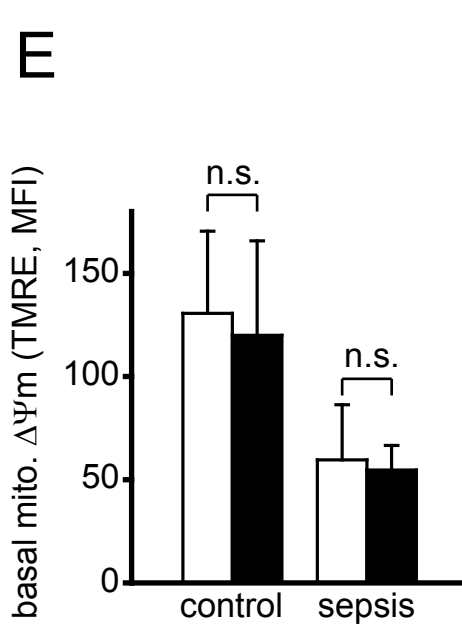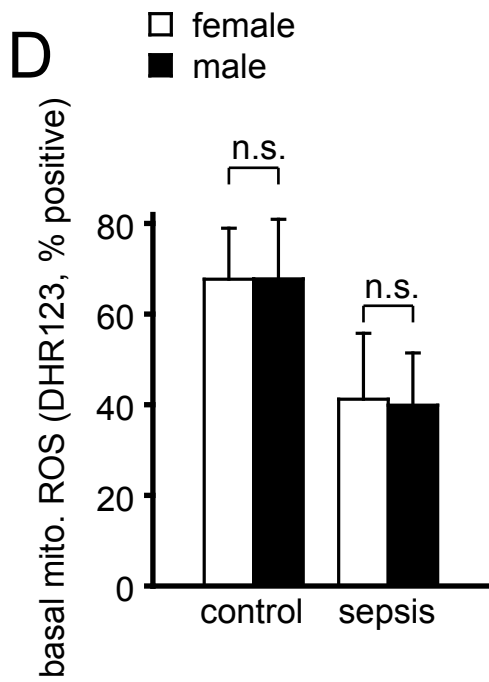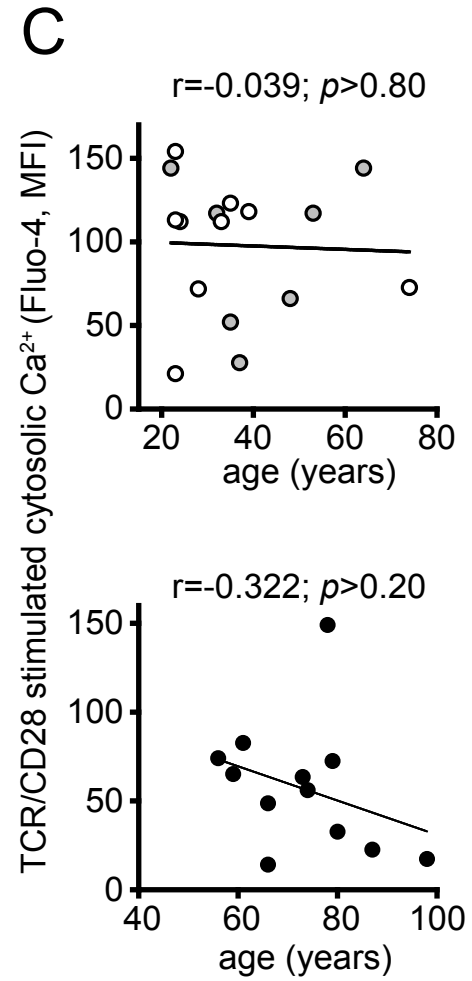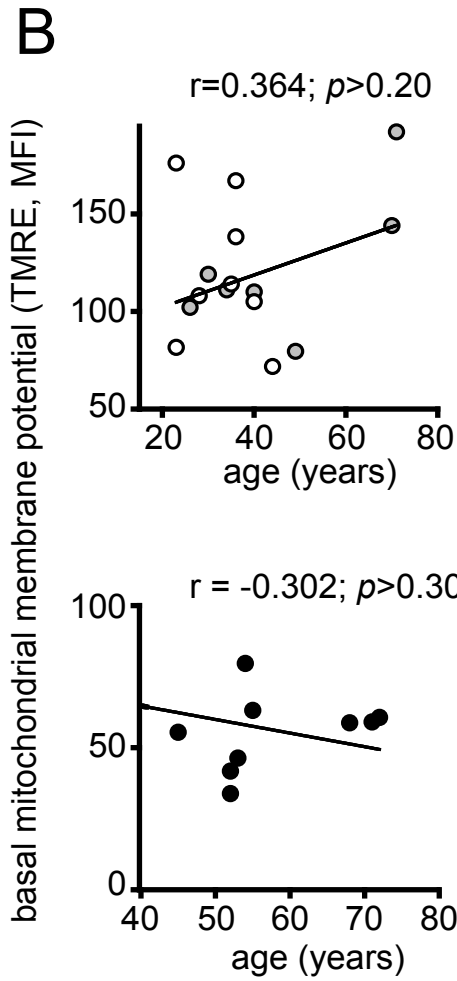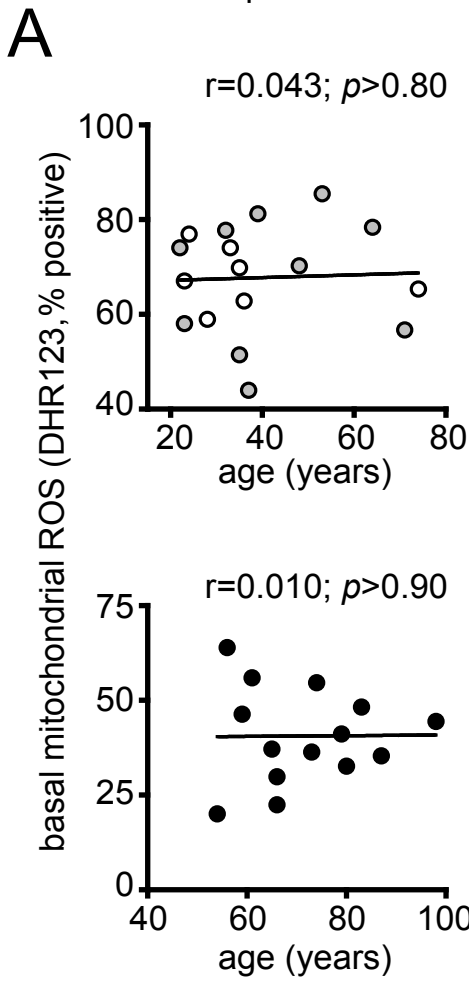
Supplementary Table 1. Characteristics of sepsis patients.

#	age	gender	sepsis syndrome	WBC (x10^9/l)	PMN (%)	lympho-cytes (%)	SOFA score	APACHEII score	out-come
1	79	F	sepsis	18.1	93.9	2.4	3	20	D
2	56	M	septic shock	19.4	88.4	5.9	2	12	A
3	61	F	severe sepsis	20.0	91.2	5.8	1	14	A
4	74	F	severe sepsis	13.0	69.2	21.4	2	13	A
5	80	M	septic shock	13.4	65.0	6.0	6	12	A
6	66	F	severe sepsis	14.1	90.3	3.0	7	14	A
7	66	M	septic shock	4.3	71.0	19.0	11	26	A
8	73	M	severe sepsis	17.1	91.8	4.0	3	18	A
9	98	M	severesepsis	4.9	81.1	13.6	4	24	D
10	83	F	septic shock	17.8	92.1	4.4	10	13	A
11	78	M	septic shock	41.1	43.0	24.0	3	14	D
12	87	M	septic shock	18.8	92.9	4.4	5	12	A
13	65	M	severe sepsis	8.9	72.1	18.7	2	9	A
14	59	F	septic shock	4.5	64.6	21.9	3	15	A
15	33	F	septic shock	9.6	84.6	12.1	11	15	A
16	72	F	septic shock	15.3	92.0	3.0	6	19	D
17	52	F	severe sepsis	16.3	90.0	2.0	0	5	A
18	45	F	sepsis	23.3	88.6	6.3	0	4	A
19	55	M	septic shock	2.6	15.0	65.0	8	21	A
20	22	F	sepsis	19.1	91.5	3.9	2	8	A
21	68	F	sepsis	14.9	92.0	3.6	1	13	A
22	52	F	severe sepsis	20.0	82.1	13.4	3	16	A
23	54	F	severe sepsis	2.4	51.6	41.5	4	9	A

M, male; F, female; WBC, white blood cells; PMN, polymorphonuclear leukocytes; SOFA score, Sequential Organ Failure Assessment score; APACHE II score, Acute Physiology, Age and Chronic Health Evaluation II score; D, dead; A, alive. Sepsis syndrome was defined according to commonly used guidelines (Levy et al., 2003).

Supplementary Table 2. Characteristics of non-septic emergency department patients.

#	age	gender	reason for presenting to the emergency department
1	23	F	gastrointestinal/gynecological
2	39	F	cardiopulmonary
3	32	F	minor traumatic injury
4	25	F	gastrointestinal
5	27	F	minor traumatic injury
6	48	M	cardiopulmonary
7	53	M	back strain
8	64	F	elbow pain
9	22	F	right ankle injury
10	23	M	minor traumatic injury
11	70	M	back pain
12	44	M	minor traumatic injury
13	71	M	malaise
14	34	F	lower back pain
15	49	M	dizziness
16	30	F	minor traumatic injury
17	26	F	cardiopulmonary
18	40	M	cardiopulmonary

M, male; F, female.