ACE-2-interacting domain of SARS-CoV-2 (AIDS) peptide suppresses inflammation to reduce fever and protect lungs and heart in mice: Implications for COVID-19 therapy

Ramesh K. Paidi[1,*], Malabendu Jana[1,*], Rama K. Mishra[2], Debashis Dutta[1], Sumita Raha[1], and Kalipada Pahan[1,3]

[1]Department of Neurological Sciences, Rush University Medical Center, Chicago, USA; [2]Department of Biochemistry and Molecular Genetics, Feinberg School of Medicine, Northwestern University, Chicago, USA; [3]Division of Research and Development, Jesse Brown Veterans Affairs Medical Center, Chicago, USA

[*]First two authors have equal contribution to the work.

Running title: AIDS peptide for COVID-19

Corresponding author:
Kalipada Pahan, Ph.D.
Department of Neurological Sciences
Rush University Medical Center
1735 West Harrison St
Suite Cohn 310
Chicago, IL 60612
Tel: (312) 563-3592; Fax: (312) 563-3571
Email: Kalipada_Pahan@rush.edu

Legend to supplementary figures:

Figure S1. Dose-dependent induction of IL-6 and IL-1β by recombinant SARS-CoV-2 spike S1 in human A549 lung cells. Human A549 lung cells were stimulated with different doses of recombinant SARS-CoV-2 spike S1 under serum-free condition for 4 h followed by monitoring the mRNA expression of IL-6 and IL-1β by semi-quantitative RT-PCR (A) and real-time PCR (B, IL-6; C, IL-1β). In parallel experiment, cells were also stimulated with boiled SARS-CoV-2 spike S1. In this case, SARS-CoV-2 spike S1 was boiled for 5 min. Results are mean $\pm$ SD of three independent experiments. $^{***}p < 0.001$.

Figure S2. Suppression of SARS-CoV-2 spike S1-mediated expression of IL-6 and IL-1β in human A549 lung cells by neutralizing antibodies against spike S1. Human A549 lung cells pretreated with different concentrations of either anti-spike S1 antibody or control IgG for 5 min were stimulated with 1 ng/ml recombinant SARS-CoV-2 spike S1 under serum-free condition for 4 h followed by monitoring the mRNA expression of IL-6 and IL-1β by semi-quantitative RT-PCR (A) and real-time PCR (B, IL-6; C, IL-1β). Results are mean $\pm$ SD of three independent experiments. $^{***}p < 0.001$.

Figure S3. Effect of wtAIDS and mAIDS peptides on the mRNA expression of IL-6 and IL-1β in SARS-CoV-2 spike S1-, poly IC-, HIV tat-, and flagellin-stimulated human A549 lung cells. Human A549 lung cells pretreated with different concentrations of wtAIDS and mAIDS peptides for 15 min were stimulated with recombinant SARS-CoV-2 spike S1 (A), polyIC (B), HIV-1 Tat- (C), and flagellin (D) under serum-free condition for 4 h followed by monitoring the mRNA expression of IL-6 and IL-1β by semi-quantitative RT-PCR. Results represent three independent experiments.

Figure S4. Schematic presentation of different cardiac parameters.

Figure S5. Intranasal delivery of wtAIDS peptide improves locomotor activities in a mouse model of COVID-19. Six-eight week old C57/BL6 mice (n=6) of both sexes were treated intranasally with wtAIDS or mAIDS peptides (100 ng/mouse/d). After 10 min, mice were intoxicated with recombinant SARS-CoV-2 spike S1 (50 ng/mouse/d) via intranasal route. After 7d of treatment, mice were tested for general locomotor activities (A, heat map; B, distance travelled; C, velocity; D, cumulative duration; E, rotorod latency). Results are mean $\pm$ SEM of six mice per group. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Figure S6. Intranasal delivery of wtAIDS peptide in a treatment paradigm improves locomotor activities in a mouse model of COVID-19. Six-eight week old C57/BL6 mice (n=5) of both sexes were treated intranasally with wtAIDS peptide (100 ng/mouse/d) from 1 d after intoxication of SARS-CoV-2 spike S1 (50 ng/mouse/d). After 7d of wtAIDS treatment, mice were tested for general locomotor activities (A, heat map; B, distance travelled; C, velocity; D, cumulative duration; E, rotorod latency). Results are mean $\pm$ SEM of five mice per group. $^{**}p < 0.01$; $^{***}p < 0.001$.

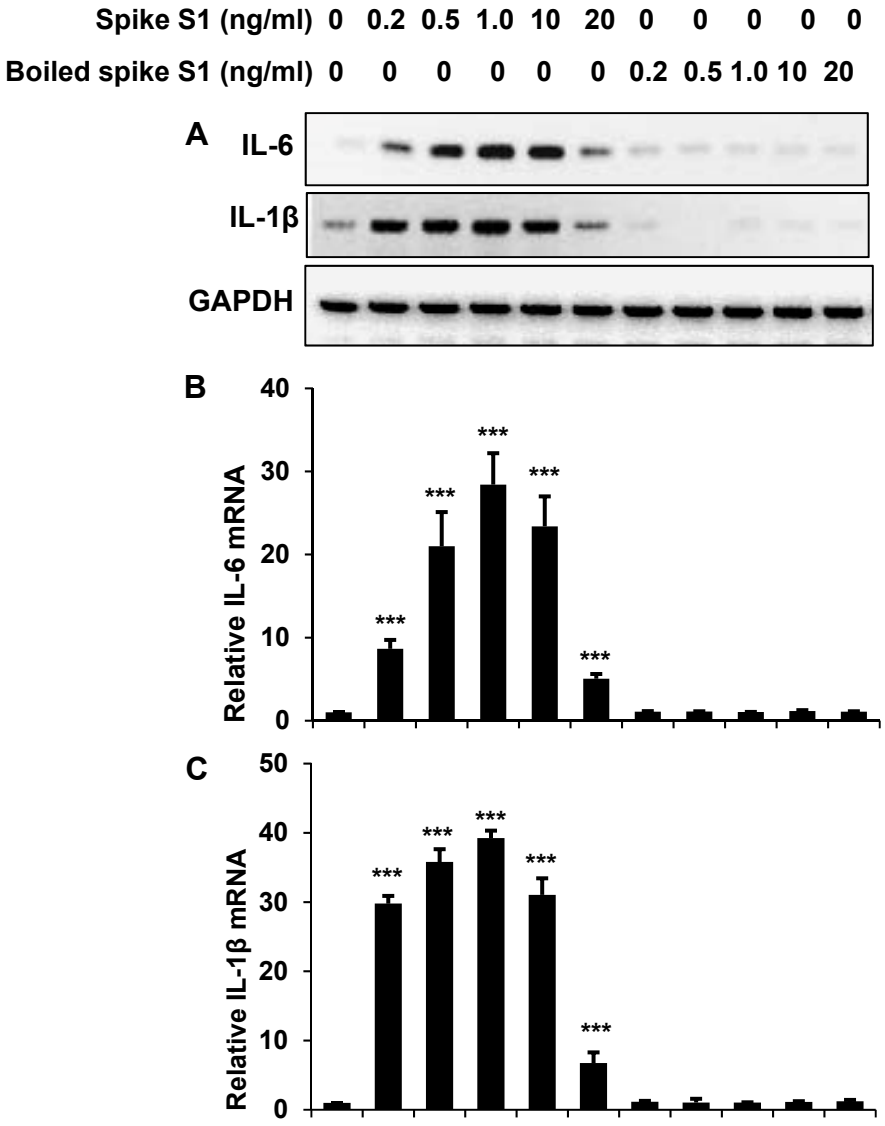
Spike S1 (ng/ml) 0 0.2 0.5 1.0 10 20 0 0 0 0 0
Boiled spike S1 (ng/ml) 0 0 0 0 0 0 0.2 0.5 1.0 10 20
A
IL-6
IL-1β
GAPDH
B
Relative IL-6 mRNA
40
30
20
10
0
***
C
Relative IL-1β mRNA
50
40
30
20
10
0
***

Figure S1

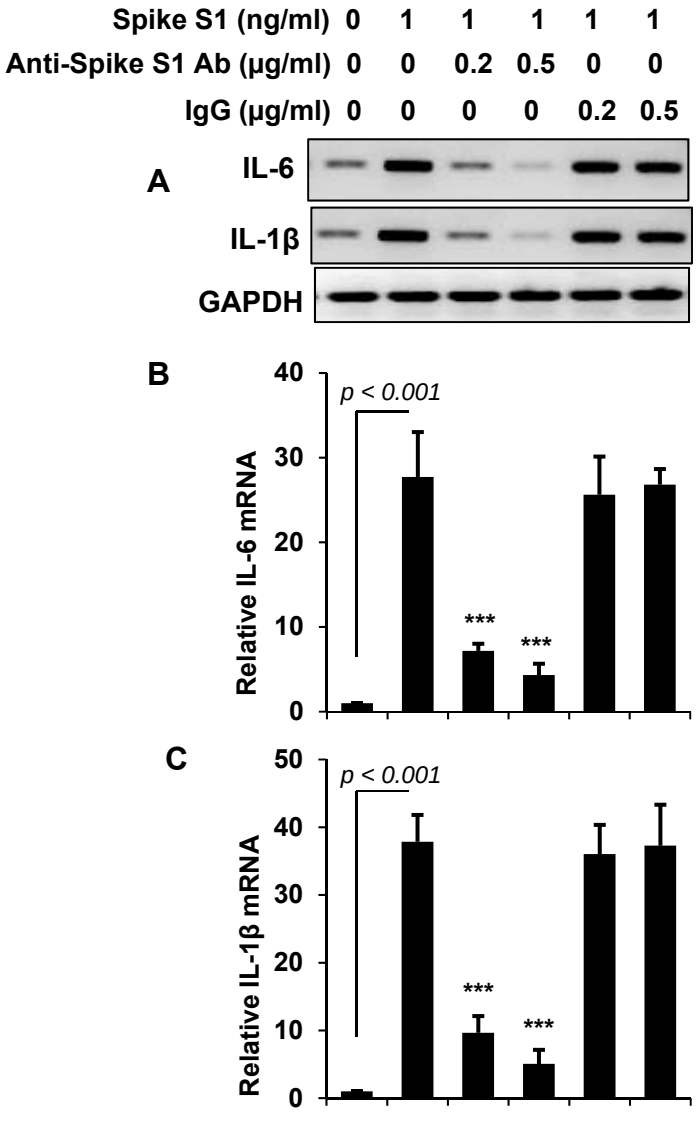
Spike S1 (ng/ml) 0 1 1 1 1 1
Anti-Spike S1 Ab (μg/ml) 0 0 0.2 0.5 0 0
IgG (μg/ml) 0 0 0 0 0.2 0.5
A
IL-6
IL-1β
GAPDH
B
Relative IL-6 mRNA
p < 0.001
***
***
C
Relative IL-1β mRNA
p < 0.001
***
***

Figure S2

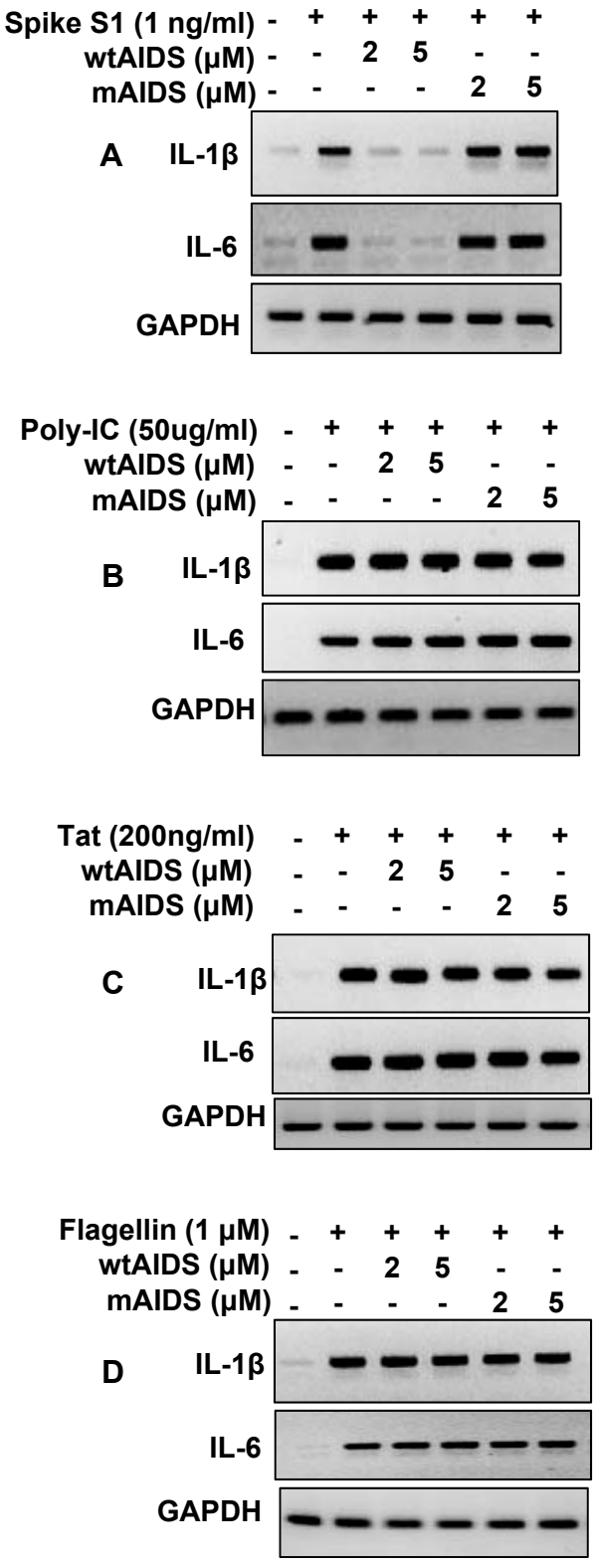
Spike S1 (1 ng/ml) - + + + + +
wtAIDS (μM) - - 2 5 - -
mAIDS (μM) - - - - 2 5
A
IL-1β
IL-6
GAPDH
Poly-IC (50ug/ml) - + + + + +
wtAIDS (μM) - - 2 5 - -
mAIDS (μM) - - - - 2 5
B
IL-1β
IL-6
GAPDH
Tat (200ng/ml) - + + + + +
wtAIDS (μM) - - 2 5 - -
mAIDS (μM) - - - - 2 5
C
IL-1β
IL-6
GAPDH
Flagellin (1 μM) - + + + + +
wtAIDS (μM) - - 2 5 - -
mAIDS (μM) - - - - 2 5
D
IL-1β
IL-6
GAPDH

Figure S3

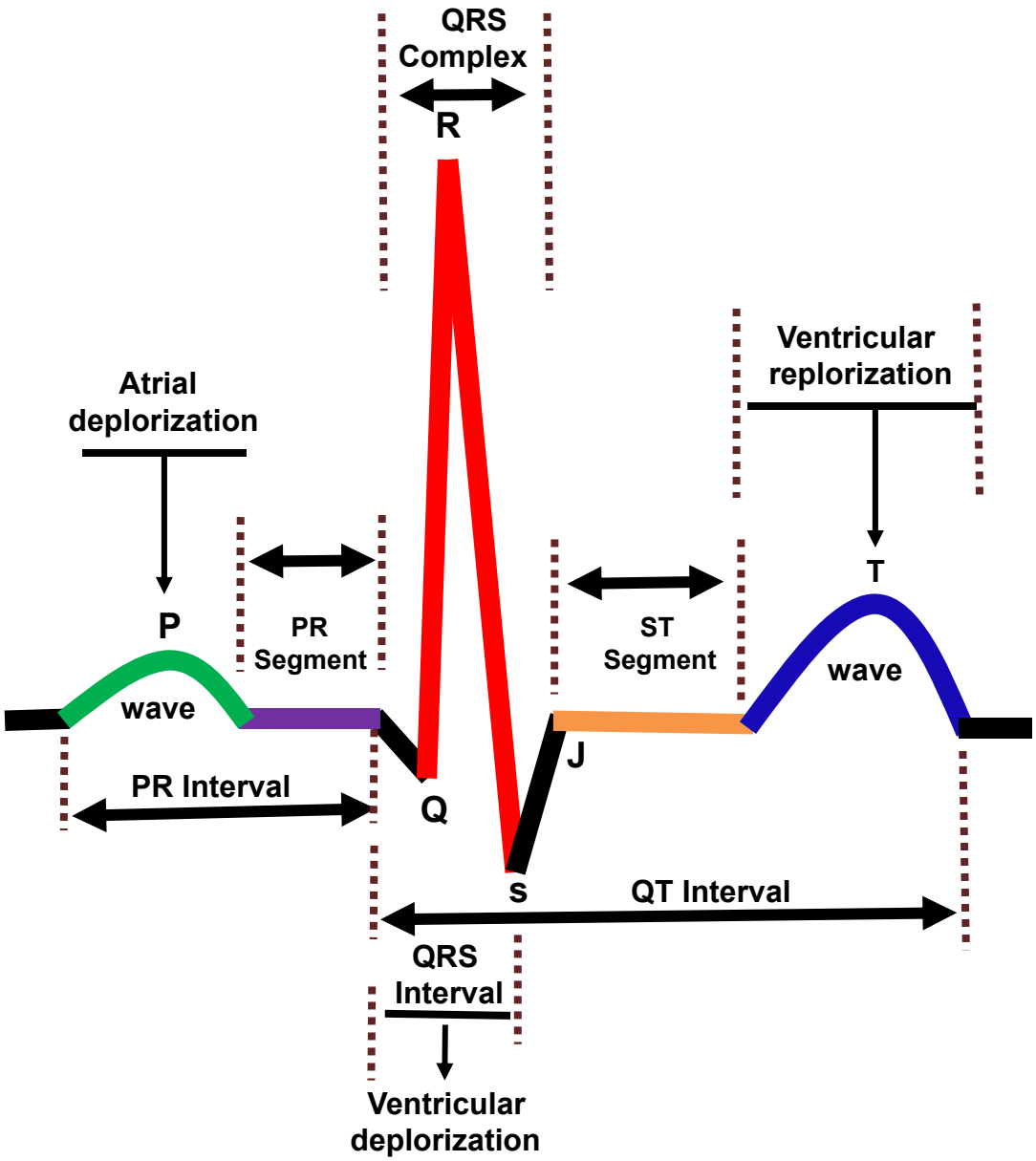
QRS
Complex
R
Atrial
deplorization
Ventricular
replorization
P
PR
Segment
ST
Segment
T
wave
wave
J
PR Interval
Q
S
QT Interval
QRS
Interval
Ventricular
deplorization

Figure S4

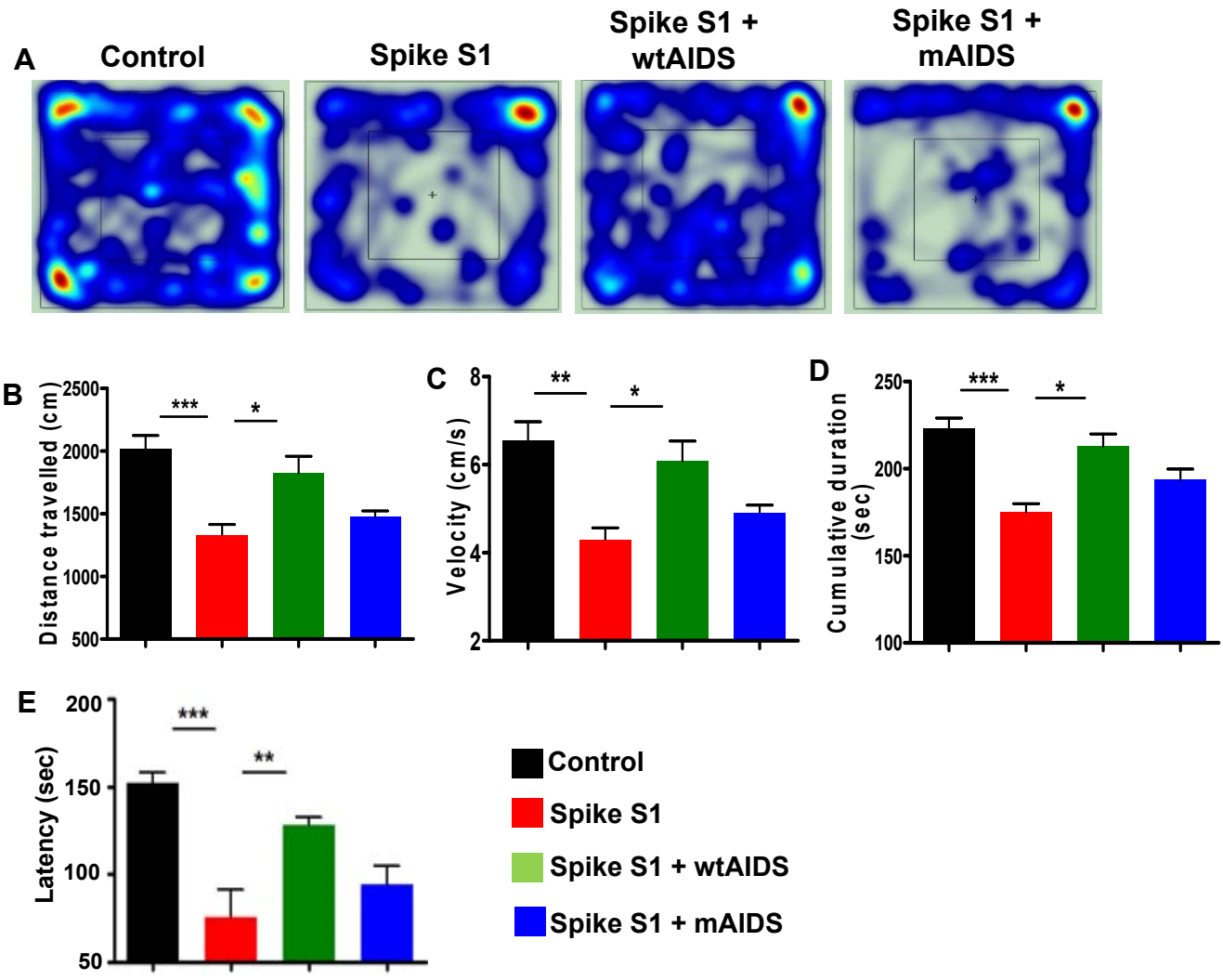
A
Control
Spike S1
Spike S1 + wtAIDS
Spike S1 + mAIDS
B
Distance travelled (cm)
2500
2000
1500
1000
500
***
*
C
Velocity (cm/s)
8
6
4
2
**
*
D
Cumulative duration (sec)
250
200
150
100
***
*
E
Latency (sec)
200
150
100
50
***
**
Control
Spike S1
Spike S1 + wtAIDS
Spike S1 + mAIDS

Figure S5

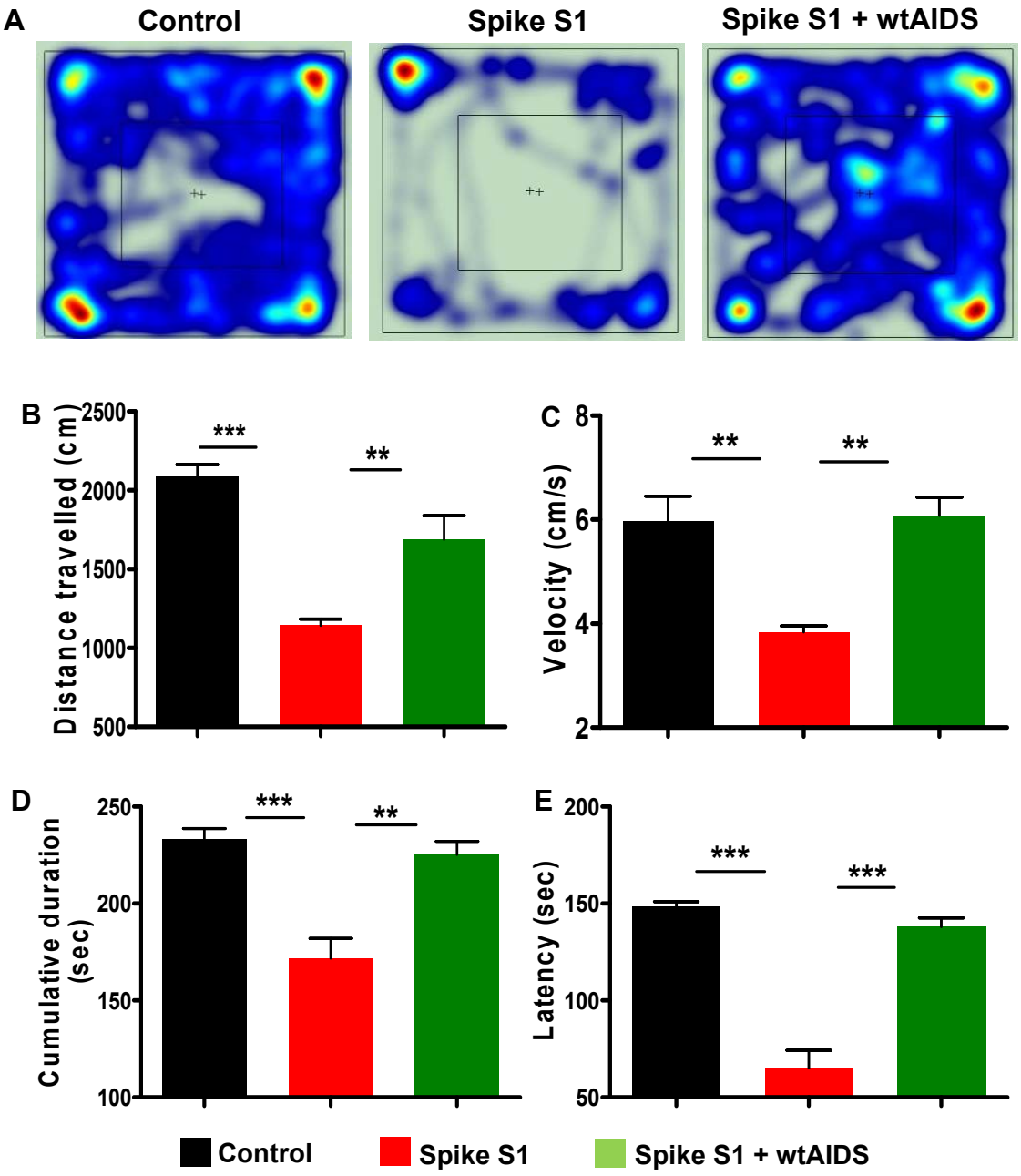
A
Control
Spike S1
Spike S1 + wtAIDS
B
Distance travelled (cm)
2500
2000
1500
1000
500
***
**
C
Velocity (cm/s)
8
6
4
2
**
**
D
Cumulative duration (sec)
250
200
150
100
***
**
E
Latency (sec)
200
150
100
50
***
***
Control
Spike S1
Spike S1 + wtAIDS

Figure S6

Table S1. Lung injury scoring system

		Score per field		
	Parameter	**0**	**1**	**2**
A.	Neutrophils in the alveolar space	None	1-5	>5
B.	Neutrophils in the interstitial space	None	1-5	>5
C.	Hyaline membranes	None	1	>1
D.	Proteinaceous debris filling the airspace	None	1	>1
E.	Alveolar Septal thickening	< 2x	2X-4X	>4x

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