

A novel peptide ACID corrects Spike S1-associated behavioral and neuronal complications in cellular and animal models.



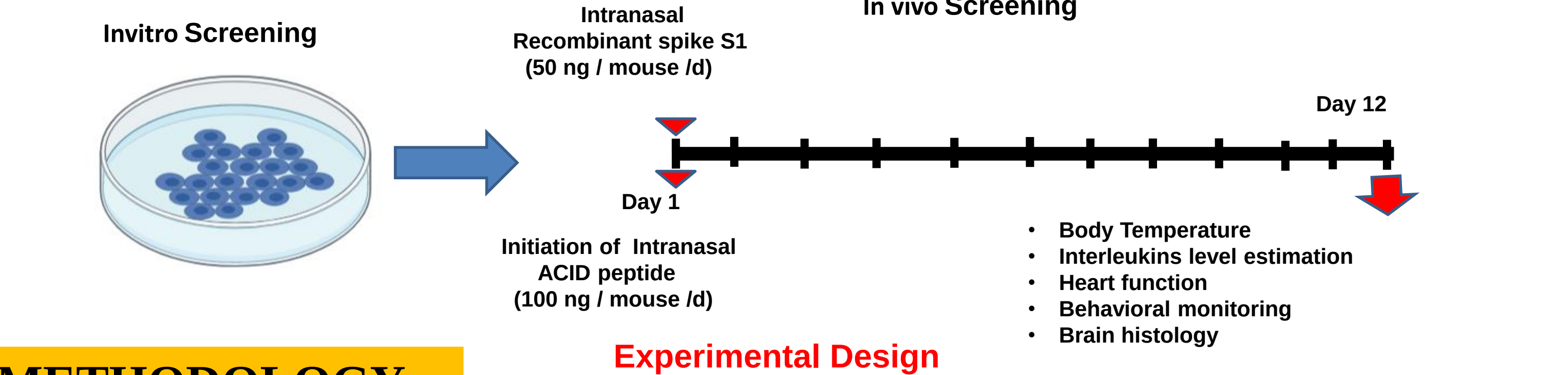
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INTRODUCTION:

- Coronavirus disease 2019 (COVID-19) is a serious infectious illness caused by the virus strain severe acute respiratory syndrome coronavirus 2 (SARS CoV-2).
- Covid-19 patients are suffered from several brain difficulties, but underlying mechanism is not clear. So far, there is no proper therapeutic strategy against Covid-19 and its complications on the brain.
- Initially we validate the effect of Spike S1 on A5339 cells and as well as mouse primary microglia in terms of cytokine induction and neuroinflammatory markers. Further, we evaluate the cytokine and neuroinflammatory levels in the mouse brain and its relevance to behavioral alterations in the mouse
- Since SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) for entering into host cells, to target COVID-19 from therapeutic angle, we designed a hexapeptide corresponding to the ACE2-interacting domain of SARS-CoV-2 (ACID) that inhibits the association between receptor-binding domain-containing spike S1 and ACE2



METHODOLOGY:

- In-vitro screening in human ACE-2 expressed HEK cells (Paidi Rk et al 2021: Paidi Rk et al 2021: Paidi Rk et al 2021), and Mouse primary microglia
- Animals: Adult (8-10 weeks old) C57BL6
- Spike S1 recombinant peptide administration (50 ng/mouse/ Day), and ACID (wt and Mt) peptide (100 ng/mouse/ Day), for 12 days of administration
- Physiological changes monitoring.
- Non-Invasive Electro cardiograph by power Lab software
- Motor and cognitive behavioural assays (open field locomotion, rotarod, Novel object recognition and Barnes maze test) for experimental animals.
- Immunofluorescence for IL6 and Iba1 in various regions of the mouse brain tissue sections.
- RT-PCR for IL6 and IL- β levels estimation in mouse brain cortical tissue

RESULTS:

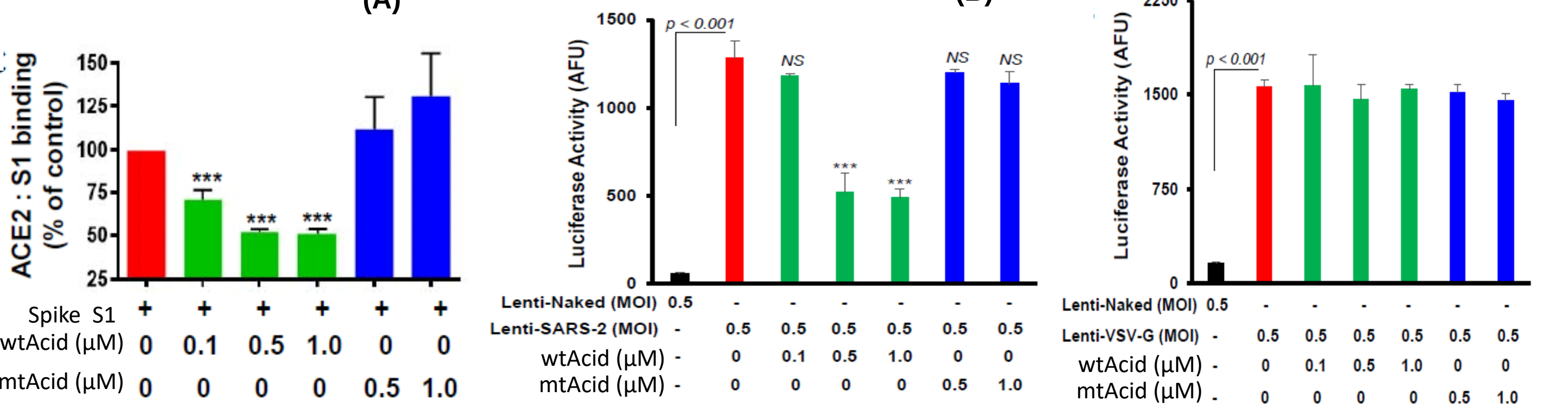


Fig 1. In vitro screening of ACID peptide A), Inhibition of ACE2 to SARSCoV-2 spike S1 binding by wtACID, but not mACID, peptide. ***p< 0.001. HEK293 cells expressing hACE2 plated at 70-80% confluence in 24-well plates were infected with lenti-naked or lentiviral particles pseudotyped with either SARS-CoV-2 spike S1 at a different MOI., after 2 d of infection, entry of pseudovirus was monitored by luciferase activity. ***p < 0.001. Postinfection with lentiviral particles, Cells pretreated with different concentrations of ACE2-interacting domain of SARS-CoV-2 (ACID) for 10 min were infected with lentiSARS-CoV- pseudovirus (MOI of 0.5). (B) After 2 d of infection, entry of pseudovirus was monitored by luciferase activity. **p < 0.01, ***p < 0.001 versus lenti-SARS-2. Cells pretreated with different concentrations of ACID for 10 min were infected with lentiVSV-G pseudovirus (MOI of 0.5). (C) After 2 d of infection, entry of pseudovirus was monitored by luciferase activity. Results represent three independent experiments. ***p < 0.001

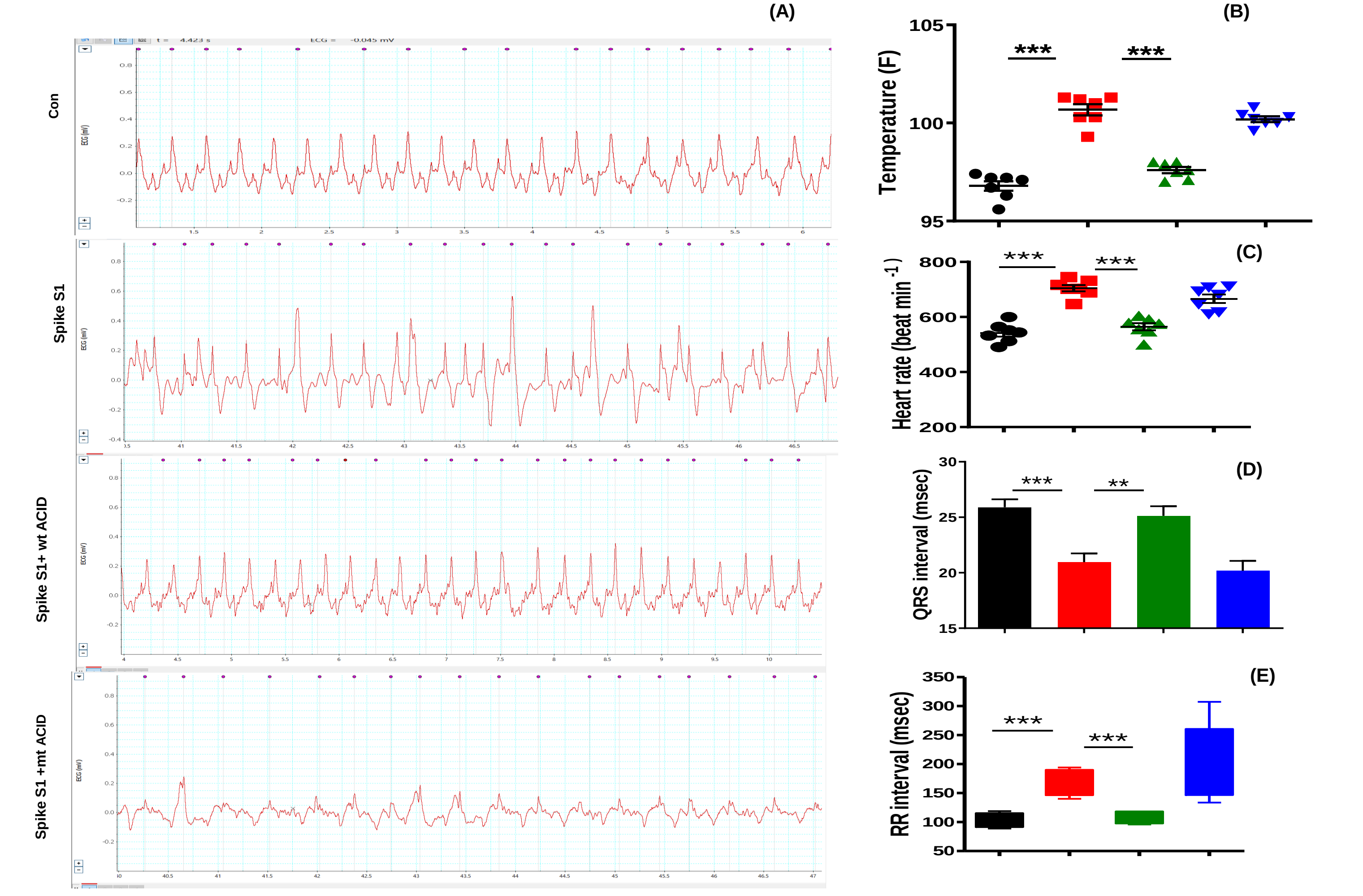


Fig 2. Effect of ACID peptide on Cardiac functions. Intranasal delivery of wt ACID peptide suppresses disease progression in a mouse model of COVID-19 but not mt ACID. Six-eight week old C57/ BL6 mice (n = 5) of both sexes were treated intranasally with wtACID/ Mt ACID peptide (100 ng/mouse/d) from 1 d after intoxication of SARS-CoV-2 spike S1 (50 ng/mouse/d). After 12d of wtACID treatment, body temperature was measured followed by monitoring heart functions by noninvasive electrocardiography (ECG) using the PowerLab (ADInstruments) (A), respective chromatograms of control mice, spike S1- intoxicated mice, (spike S1 + wtACID)-treated mice spike S1- intoxicated mice, (spike S1 + mtACID)-treated mice B, body temperature; C, heart rate; D, QRS interval E; RR interval; H, heart rate variability; F, Schematic representation of Cardiogram].***p < 0.001

Motor Functions

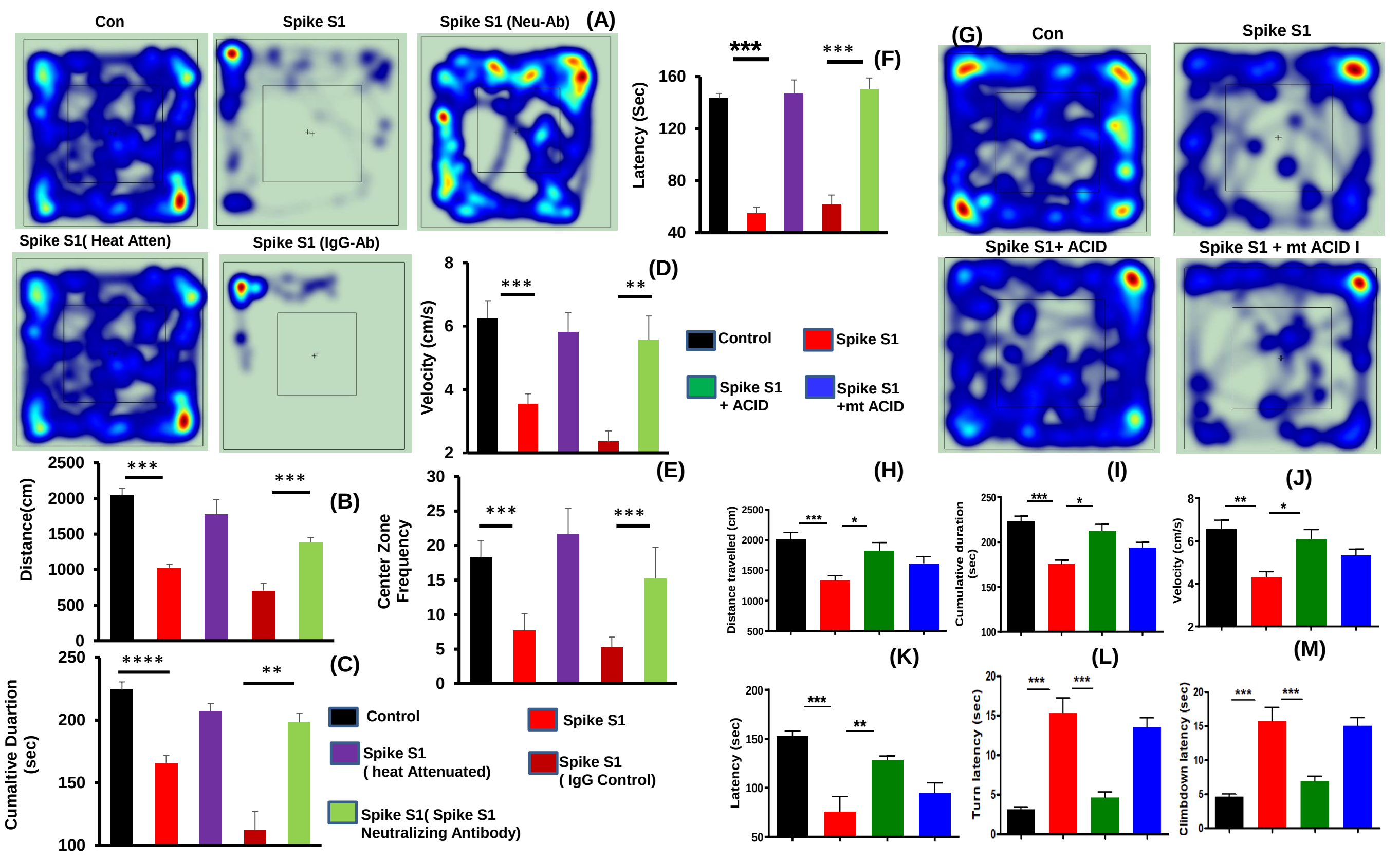


Fig 3. Specificity of SARS-CoV-2 spike S1-mediated mouse model of COVID-19. Six-eight-week-old C57/BL6 mice (n=6) of both sexes were intoxicated with recombinant SARS-CoV-2 spike S1 (50 ng/mouse/d) via intranasal route. To understand the specificity, one group of mice received same amount of boiled SARS-CoV-2 spike S1. Other groups of mice received the combination of SARS-CoV-2 spike S1 (50 ng) and 200 ng of either anti-spike S1 antibody or control IgG. Mice were tested for general locomotor activities (A), heat map; B, distance travelled; C, Cumulative duration D, velocity; E, center zone frequency; F, rotarod latency). Results are mean + SEM of 6 mice per group. ***p < 0.001. **Effect of ACID petide on Motor functions** . Mice were tested for general locomotor activities (G), heat map; H, distance travelled; I, Cumulative duration J, velocity; K, rotarod latency L, pole test turn latency.M, Climb down latency. Results are mean + SEM of 6 mice per group. ***p < 0.001.

Cognitive Functions

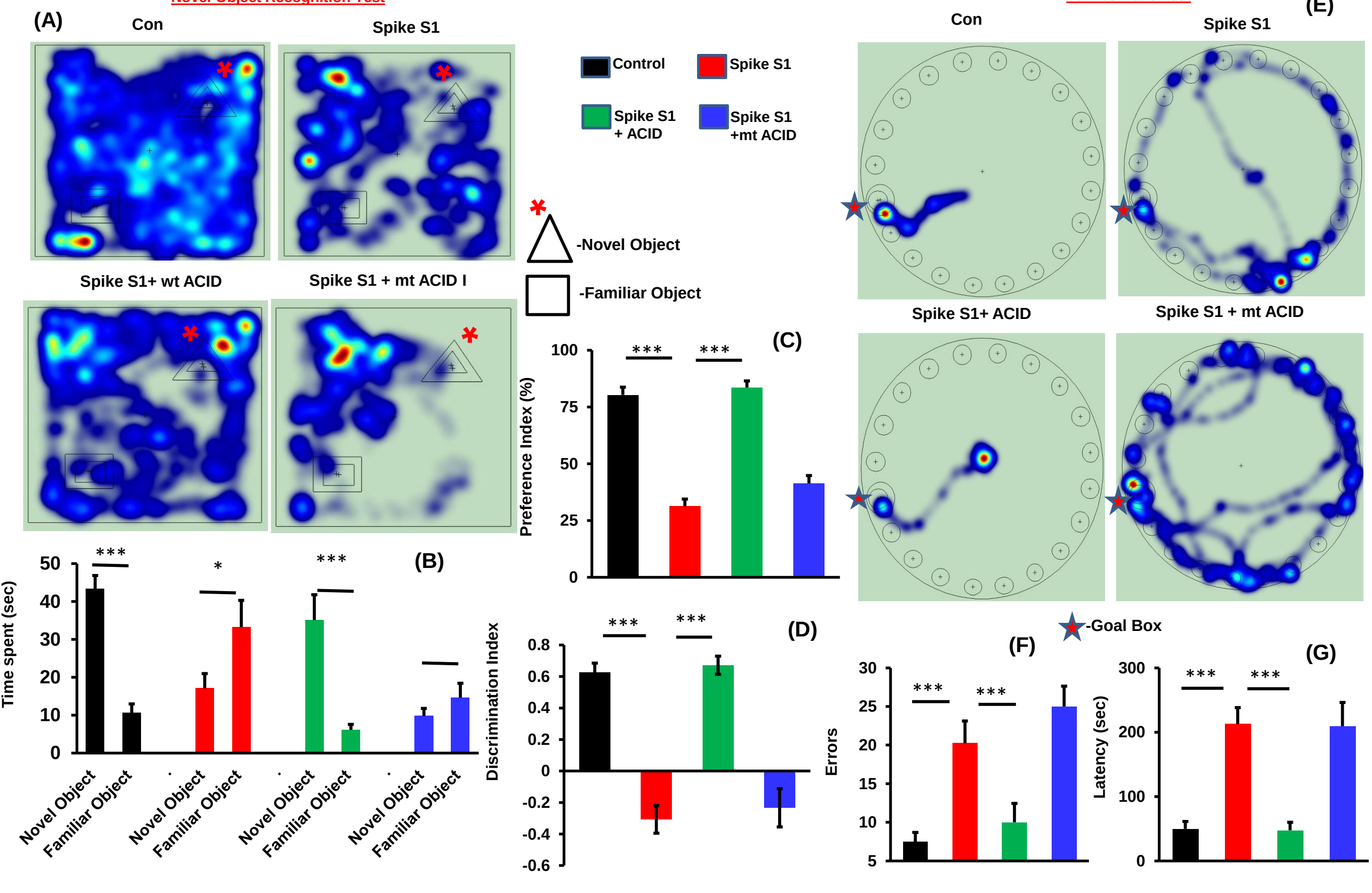


Fig 4. Effect of ACID peptide on Cognitive functions. NOR, Mice were tested for Novel object recognition memory test for short term memory (a), heat maps for respective treatment groups ; B Time spent at novel object and familiar objects ; C, Preference Index D, Discrimination index ; Results are mean + SEM of 6 mice per group. ***p < 0.001 (n=5 animals per group) . **BMT**, Mice were tested for Barnes maze cognitive test for long term memory (E), heat maps for respective treatment groups ; F Errors on probe day ; G, Latency to goal box in probe day. Results are mean + SEM of 6 mice per group. ***p < 0.001(n=5 animals per group).

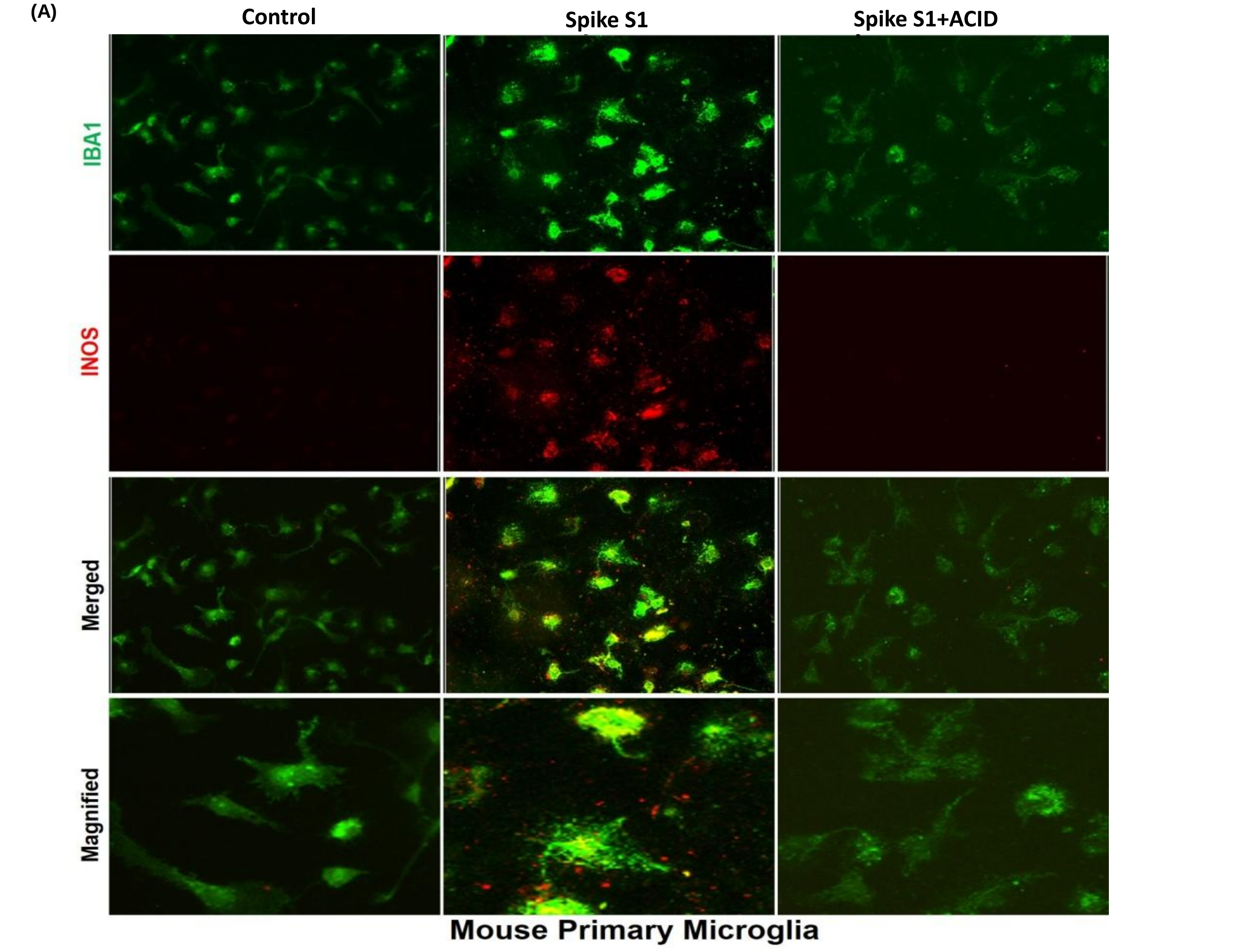


Fig 5. Effect of ACID peptide on Spike S1 induced IBA1 activation and INOS expression in Mouse primary microglia. Mouse primary Microglia was isolated from WT mice were treated with Spike S1 (1 ng/ml) followed by Wild type ACID peptide (5 μ M) or PBS A, IBA1 and INOS fluorescence were recorded in an Olympus BX-41 fluorescence microscope at 20 x magnification. An increase in INOS expression and activation of microglia was observed after Spike S1 treatment compared to vehicle treated microglia was significant. Wt ACID significantly attenuates Spike S1 induced INOS and Iba1 activation in microglia. Data were compared to Vehicle-treated WT control and represented and Mean fluorescence intensity B, IBA 1 CTCF value C, INOS CTCF Value D, Mean Iba1 positive INOS Cells . Results are mean $\pm$ SEM of three experiments. **p<0.001.

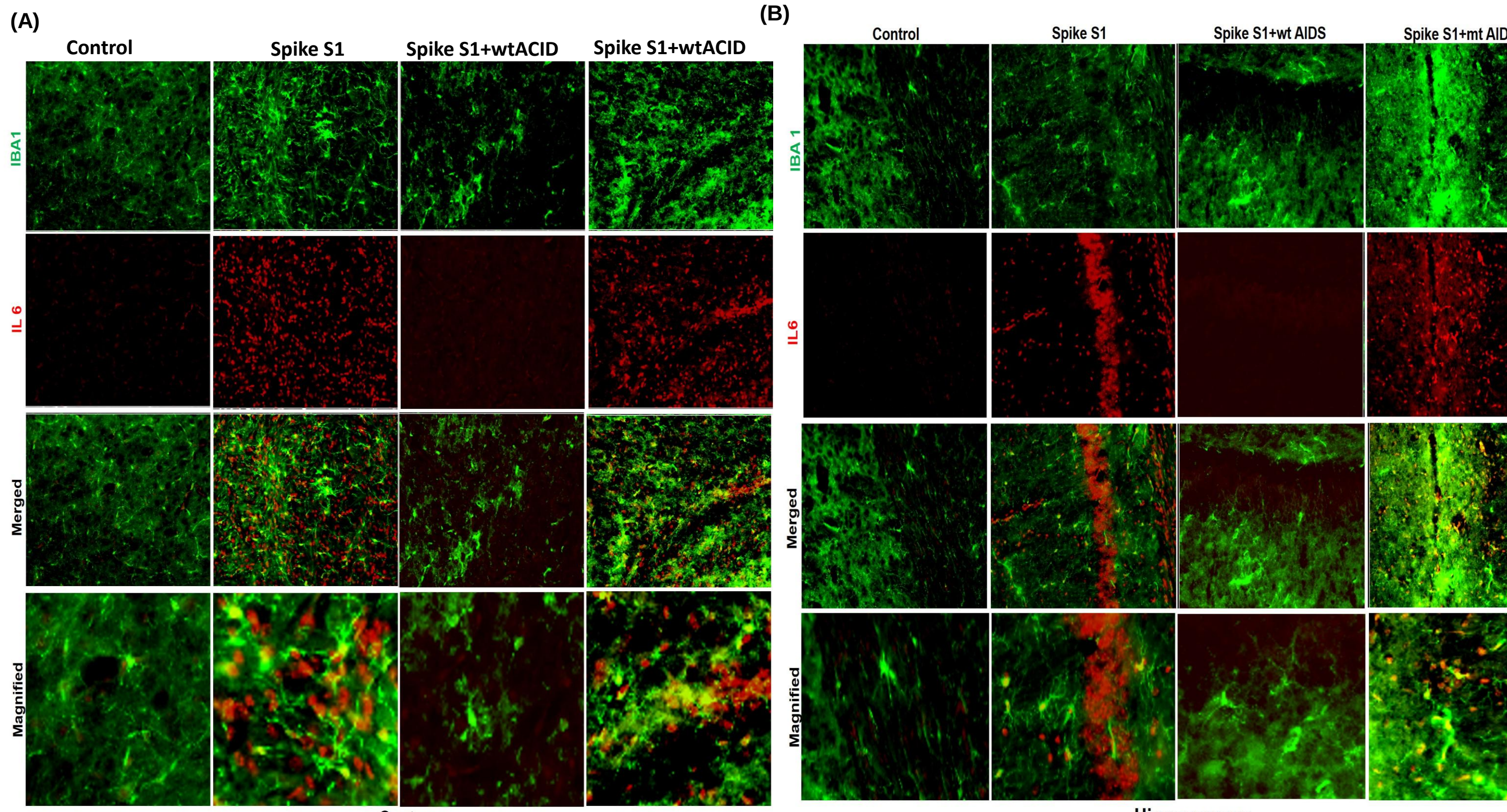


Fig. 6. ACID Peptide attenuates Spike S1 induced IL6 and Iba1 expression in different regions of the brain . Six-week-old C57/ BL6 mice (n = 5) of both sexes were treated intranasally with wt ACID peptide/Mutant ACID (100 ng/mouse/d) from 1 d after intoxication of SARS-CoV-2 spike S1 (50 ng/mouse/d). After 12d of wtACID treatment, (A) Immunofluorescence pictures show activation of IBA1 and IL6 expression was observed in In Cortex (A), Hippocampus (B), Striatum (C) after Spike S1administration in wild type mice. Wild type ACID attenuates Spike S1 induced IL6 and Iba1 expression in different regions of the brain but not mutant ACID. IL 6 and Iba1 MFI was calculated (Data was not shown) (D, E) Real time PCR was employed to estimate the levels of IL 6 and IL β expression in the brain cortical tissue . Data are represented as mean $\pm$ SEM (n=5 animals per group).

SUMMARY AND CONCLUSION :

- Spike S1 treatment prominently elevate the cytokine levels and inflammatory markers in in vitro models.
- Intranasal intoxication of C57/BL6 mice with recombinant SARS-CoV-2 spike S1 led to fever, increase in IL-6 in the lungs and brain
- We found that the impairment in spatial learning memory functions and motor disabilities, mimic some of the important symptoms of COVID-19. Our studies show Spike S1-induced cytokine levels in the lungs and in the brain.
- The wt ACID peptide targets only SARS CoV-2 – dependent Pathway without inhibiting normal physiological functions of ACE-2
- Spike S1 induced Cardiac alterations and Lung infiltration was inhibited by wt ACID
- The Wt ACID peptide Improves motor and exploratory function in Spike S1 intoxicated Mice
- Cognitive disabilities in Spike S1 mice were protected by intranasal wt ACID treatment
- The intranasal wt ACID halts the inflammatory and cytokine induction in spike S1 administered mice brain
- In summary, Our results suggest a simple, but reliable means of developing a Spike S1 mediated in vivo model which exhibits severe lung pathology and neuro-complications hence reinforce the validity of this animal model to study COVID-19 pathogenesis and potential therapies

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