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Infection with *Toxoplasma gondii* triggers coagulation at the blood-brain barrier and a reduction in cerebral blood flow

Evelyn M. Hoover[1,2], Christine A. Schneider[1,2], Christian Crouzet[3,4†], Tatiane S. Lima[5†], Dario X. Figueroa Velez[6,7], Cuong J. Tran[1,2], Dritan Agalliu[10,11], Sunil P. Gandhi[6], Bernard Choi[3,4,8,9] and Melissa B. Lodoen[1,2*]

Abstract

Background Immunothrombosis is the process by which the coagulation cascade interacts with the innate immune system to control infection. However, the formation of clots within the brain vasculature can be detrimental to the host. Recent work has demonstrated that *Toxoplasma gondii* infects and lyses central nervous system (CNS) endothelial cells that form the blood-brain barrier (BBB). However, little is known about the effect of *T. gondii* infection on the BBB and the functional consequences of infection on cerebral blood flow (CBF) during the different stages of infection.

Main body We demonstrate that brain endothelial cells upregulate the adhesion molecules ICAM-1 and VCAM-1 and become morphologically more tortuous during acute *T. gondii* infection of mice. Longitudinal two-photon imaging of cerebral blood vessels during infection in mice revealed vascular occlusion in the brain, prompting an analysis of the coagulation cascade. We detected platelet-fibrin clots within the cerebral vasculature during acute infection. Analysis of CBF using longitudinal laser-speckle imaging during *T. gondii* infection demonstrated that CBF decreased during acute infection, recovered during stable chronic infection, and decreased again during reactivation of the infection induced by IFN-γ depletion. Finally, we demonstrate that treatment of mice with a low-molecular-weight heparin, an anticoagulant, during infection partially rescued CBF in *T. gondii*-infected mice without affecting parasite burden.

Conclusions Our data provide insight into the host-pathogen interactions of a CNS parasite within the brain vasculature and suggest that thrombosis and changes in cerebral hemodynamics may be an unappreciated aspect of infection with *T. gondii*.

Keywords *Toxoplasma gondii*, CNS infection, Thrombosis, Cerebral blood flow, Blood-brain barrier

†Christian Crouzet and Tatiane S. Lima contributed equally to this work.

*Correspondence:
Melissa B. Lodoen
mlodoen@uci.edu

Full list of author information is available at the end of the article

Background

E. coli, *Yersinia enterocolitica*, *Plasmodium falciparum*,

Immuno□uorescence staining, imaging, and analysis

Serum cytokine analysis

2-Photon imaging

Endothelial cell morphology analysis

T. gondii.

$$\text{shape index} = \frac{4\pi * \text{area}}{(\text{perimeter})^2},$$

Laser speckle imaging

$$\text{CBF} = \frac{1}{2TK^2},$$

Low-molecular-weight heparin treatment

Fibrin extraction and western blotting

Flow cytometry

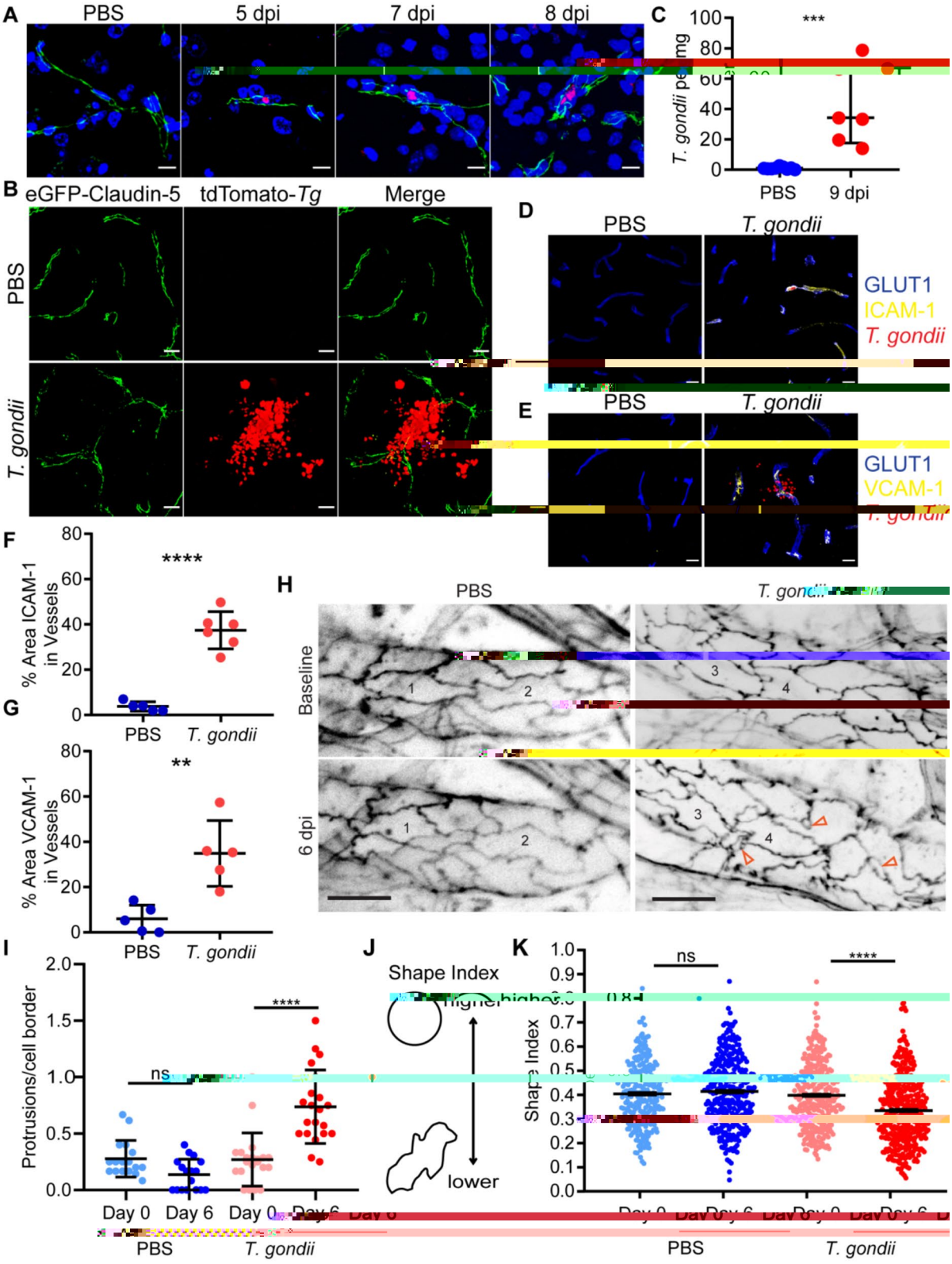

Fig. 1 (See legend on next page.)

Statistics

Results

Effect of acute *T. gondii* infection on CNS vascular endothelium

T. gondii **infection induces clotting in the brain microvasculature**

Hemodynamic changes during *T. gondii* infection

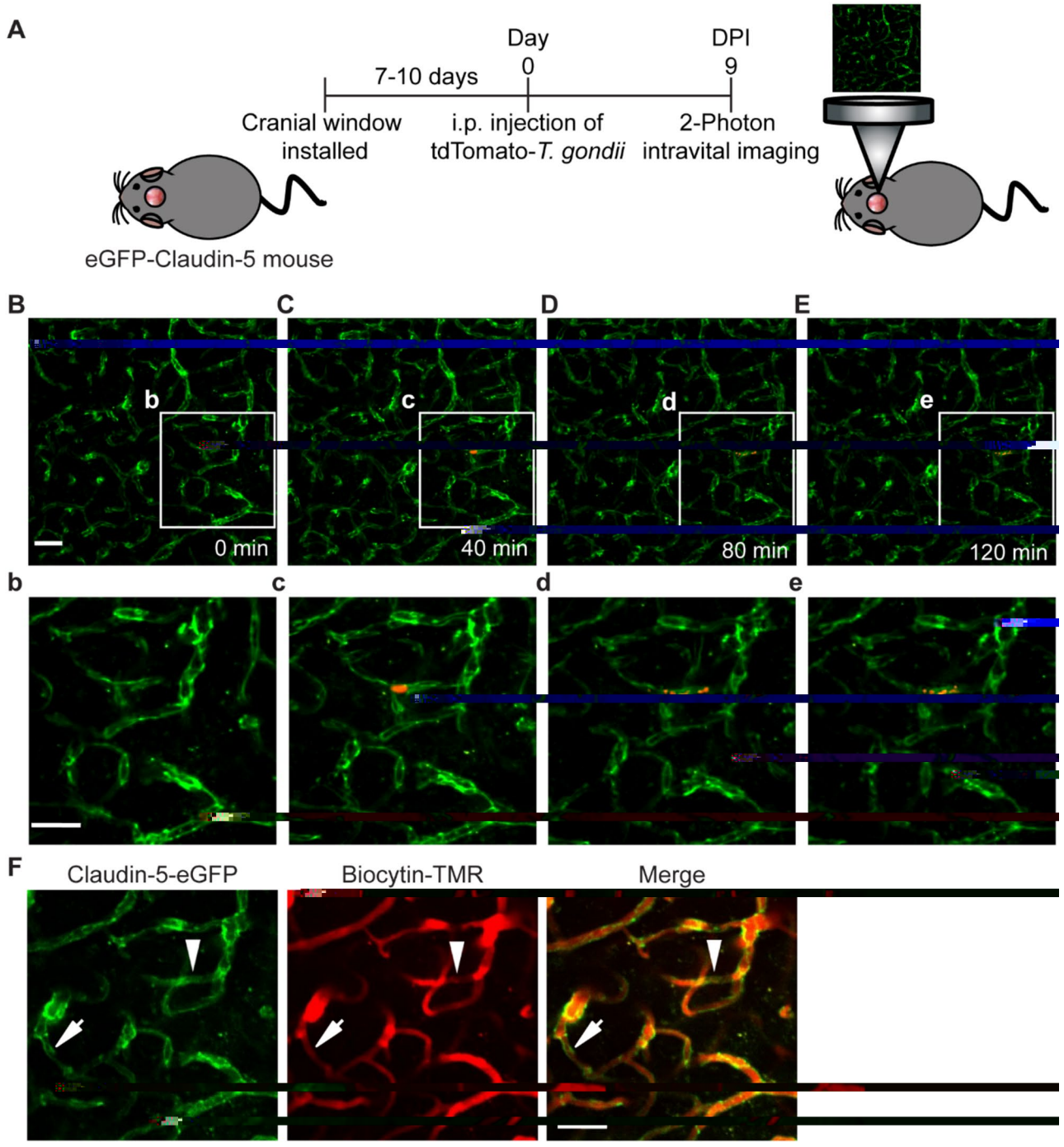

Fig. 2 Reduced blood vessel perfusion during acute *T. gondii* infection. **A**) Schematic of experimental work□ow for intravital 2-photon imaging in the cortex of an eGFP-Claudin-5 mouse infected with tdTomato-expressing *T. gondii* at 9 dpi. **B-E**) Imaging in the same □eld of view (FOV) at 0, 40, 80, and 120 min. **b-e**) Insets show a magni□ed FOV around the *T. gondii* vacuole. **F**) Biocytin-TMR (860 D) was injected i.v. during imaging and dye perfusion (red) was imaged in the same FOV as in **B-E**. Arrowhead shows the reduced dye perfusion adjacent to the *T. gondii* parasites. Arrow shows a location distal to the parasites with reduced perfusion. Scale bars, 50 µm

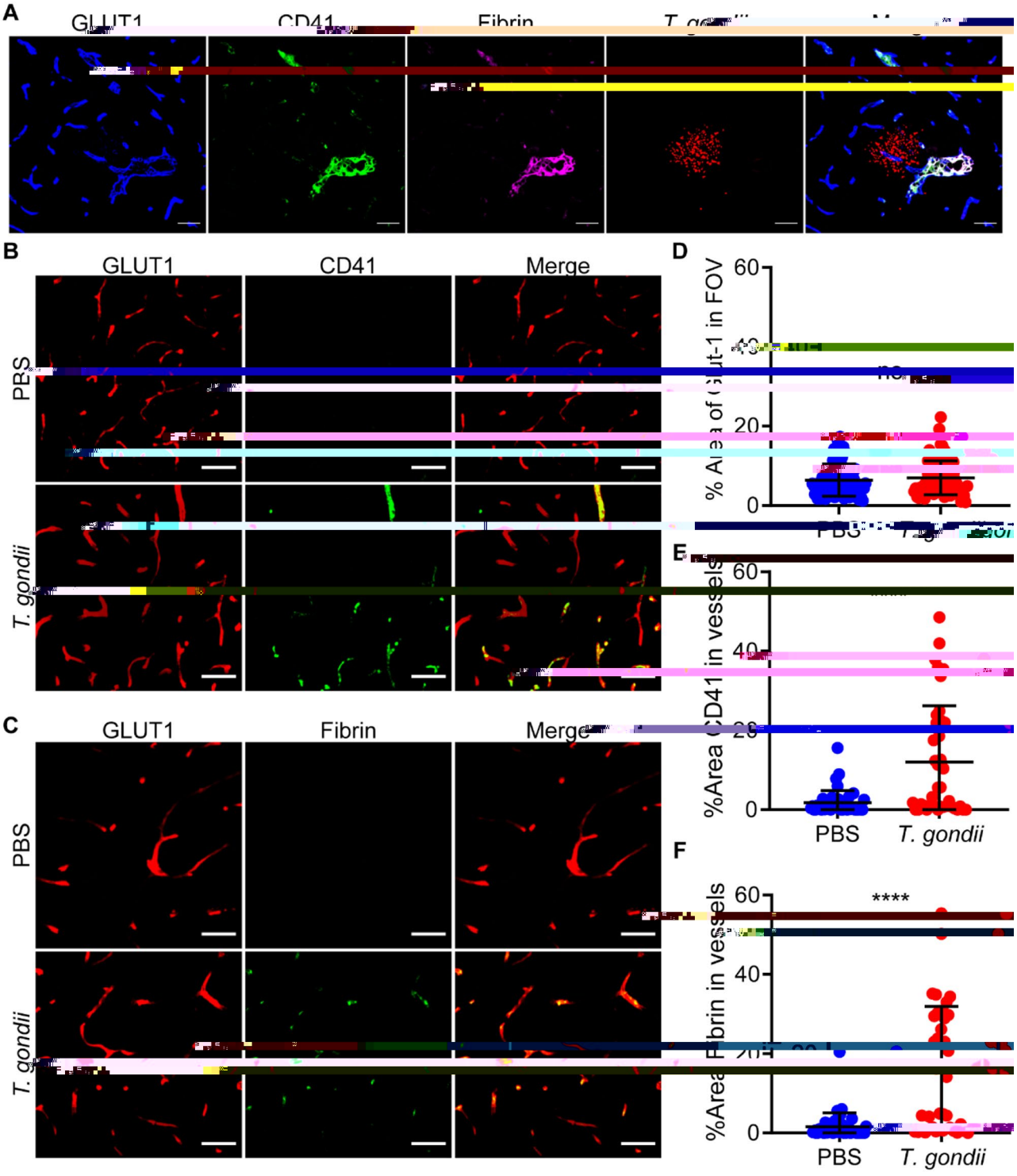

Fig. 3 Evidence of platelet-fibrin clot formation in cerebral vessels of *T. gondii*-infected mice. C57BL/6J mice were infected with *T. gondii* or injected with PBS, and brains were harvested at 9 dpi. **A**) Confocal image of brain section stained with antibodies against GLUT1, CD41, and fibrin. Scale bars, 50 µm. **B-C**) Widefield images of brain sections stained with anti-GLUT1 and either anti-CD41 (**B**) or anti-fibrin (**C**). Scale bars, 50 µm. **D**) Percent area of GLUT1 within each FOV. **E-F**) Percent area of CD41 or fibrin in GLUT1$^+$ vessels, respectively. Each circle represents one FOV. $n = 40–80$ FOVs from 4 independent mice per group. **** $P < 0.0001$; Student's t test. Error bars represent SD

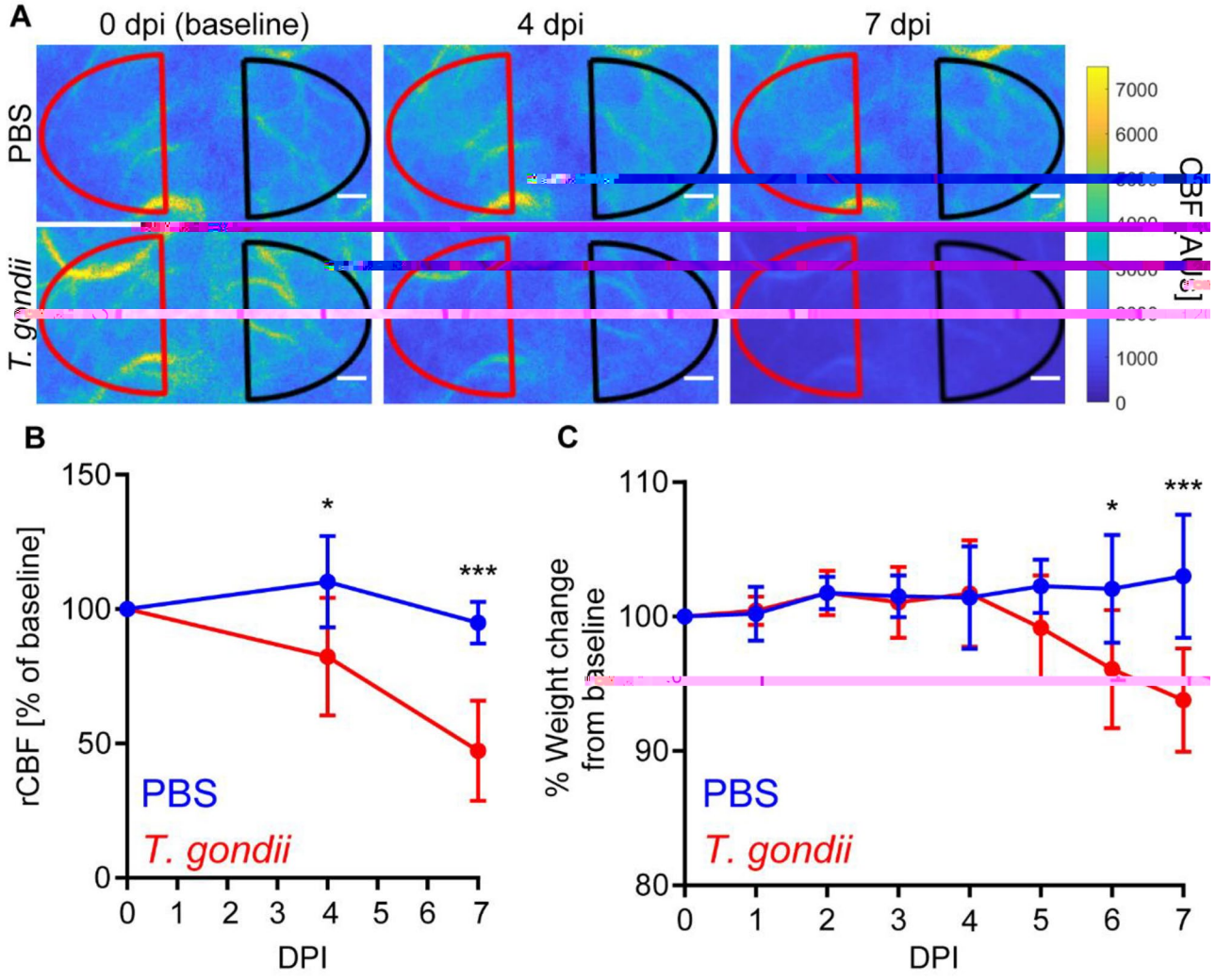

Fig. 4 CBF changes during acute *T. gondii* infection. C57BL/6 mice were injected with PBS or infected with *T. gondii* and longitudinal laser speckle imaging was performed at 0, 4, and 7 dpi through the intact skull to measure CBF. **A**) Representative laser speckle images of control and *T. gondii*-infected mice. Scale bars, 400 µm. **B**) Percent change of rCBF to baseline in control and *T. gondii*-infected mice. **C**) Percent weight change from baseline in control and *T. gondii*-infected mice. $n = 4–5$ mice per group. $^{*}P < 0.05$, $^{***}P < 0.001$; signi□cance between mock and infected mice at each timepoint was calculated by a repeated measures two-way ANOVA, with a post hoc Sidak's multiple comparisons test. Error bars represent SD

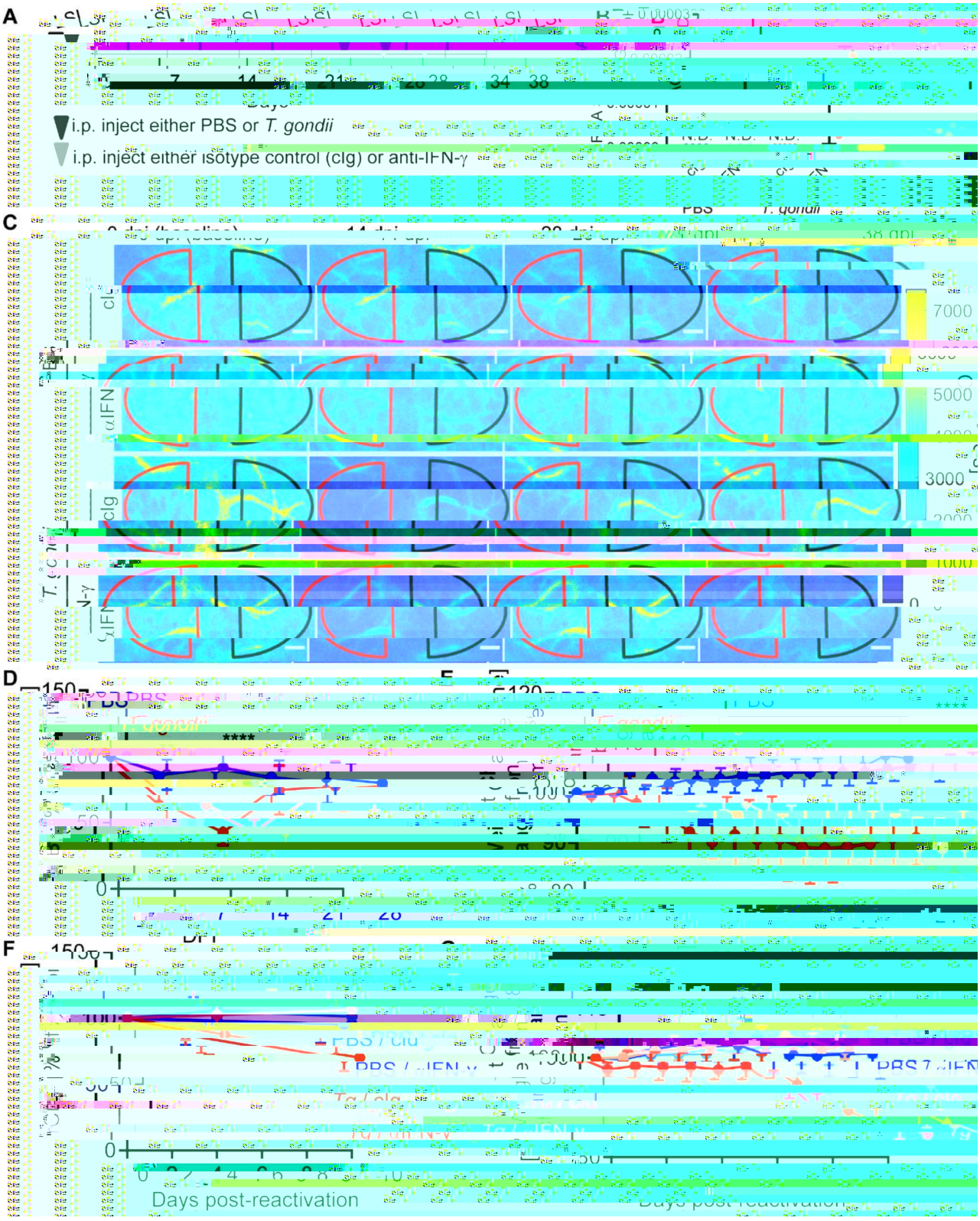

Fig. 5 (See legend on next page.)

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Fig. 5 CBF changes during chronic *T. gondii* infection. **A**) Experimental set-up for chronic infection and reactivation laser speckle imaging (LSI) experiments. C57BL/6 mice were injected with PBS or infected with 200 type II *T. gondii* tachyzoites at 0 dpi after the □rst imaging session. Antibodies were injected at 28 and 32 dpi. **B**) Relative expression of SAG1 to GAPDH transcripts in brains of control (PBS) and *T. gondii*-infected mice at 38 dpi. Mice were administered control IgG (cIg) or anti-IFN-γ (αIFN-γ) starting at 28 dpi. SAG1 was not detected (ND) in any group except the *T. gondii*-infected mice given anti-IFN-γ. **C**) Representative laser speckle imaging showing CBF in control (PBS) or *T. gondii*-infected mice at 0, 14, 28, and 38 dpi. The red and black hemispheres show the ROIs for the right and left hemispheres. Scale bars, 400 μm. **D**) Percent change of rCBF to baseline in control and *T. gondii*-infected mice over time. **E**) Percent weight change from baseline in control and *T. gondii*-infected mice. (**F**) Percent change of rCBF in control and *T. gondii*-infected mice (*Tg*) administered cIg or anti-IFN-γ. (**G**) Percent weight change in control and *T. gondii*-infected mice administered cIg or anti-IFN-γ during reactivation. $n = 8–11$ mice (**D**-**E**) and 4–7 mice per group (**F**-**G**). *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, ****$P < 0.0001$; di□erences between groups at each timepoint were determined by a two-way ANOVA (**D**-**E**) or a mixed-e□ects analysis (**F**-**G**) with a post-hoc Sidak's multiple comparisons test. In **F**-**G**, signi□cance is shown between *T. gondii*-infected mice treated with IgG or anti-IFN-γ. Error bars represent SD

Discussion

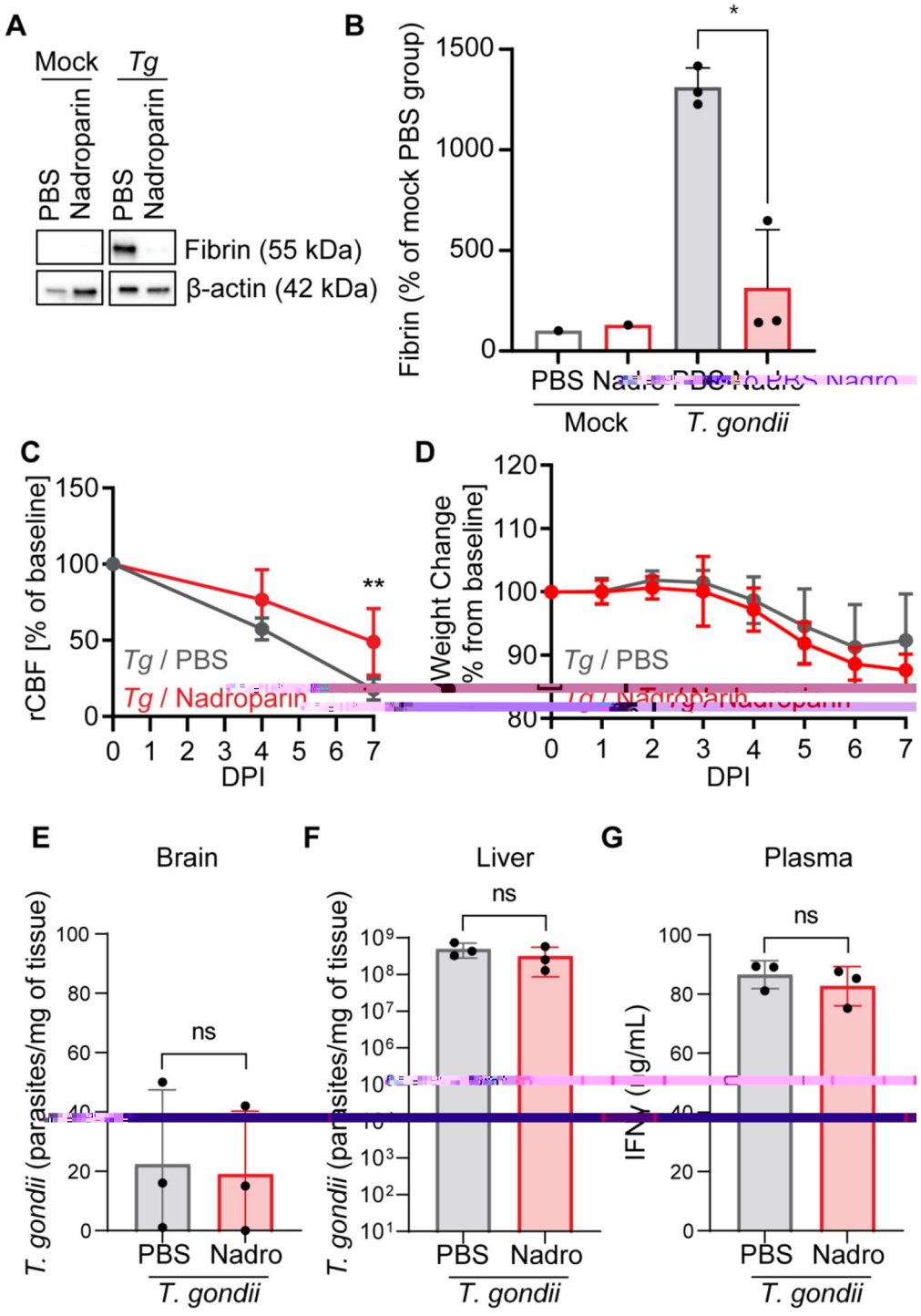


Fig. 6 Nadroparin calcium treatment of *T. gondii*-infected mice. **A**) Representative Western blot of fibrin and β-actin from livers of mock- and *T. gondii*-infected mice treated with PBS or Nadroparin at 7 dpi. **B**) Quantification of fibrin in mock-treated mice given PBS or nadroparin and *T. gondii*-infected mice given PBS or nadroparin. $n = 1–3$ mice per group. * $P < 0.05$; significance was calculated by one-way ANOVA followed by a Tukey post-test. **C**) Percent change of rCBF to baseline in *T. gondii*-infected mice treated with PBS or nadroparin calcium at 0, 4, and 7 dpi. **D**) Percent weight change from baseline in *T. gondii*-infected mice treated with PBS or nadroparin calcium. $n = 3–6$ mice per group. *$P < 0.05$, **$P < 0.005$, ns = not significant; significance was calculated by a mixed-effects analysis with a post hoc Sidak's multiple comparisons test. Brain (**E**) and liver (**F**) homogenates were examined for the *T. gondii* B1 gene by qPCR to determine *T. gondii* per mg of tissue in *T. gondii*-infected mice treated with PBS or nadroparin (7 dpi). **G**) IFN-γ levels were quantified with an ELISA in *T. gondii*-infected mice treated with PBS or nadroparin (7dpi). $n = 3$ mice per group. Significance was calculated with a Student's *t* test. Error bars represent SD

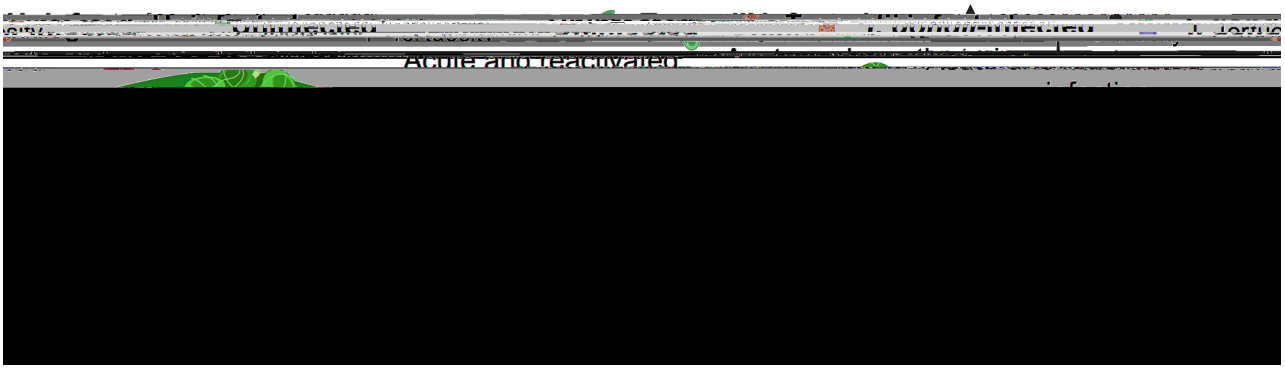
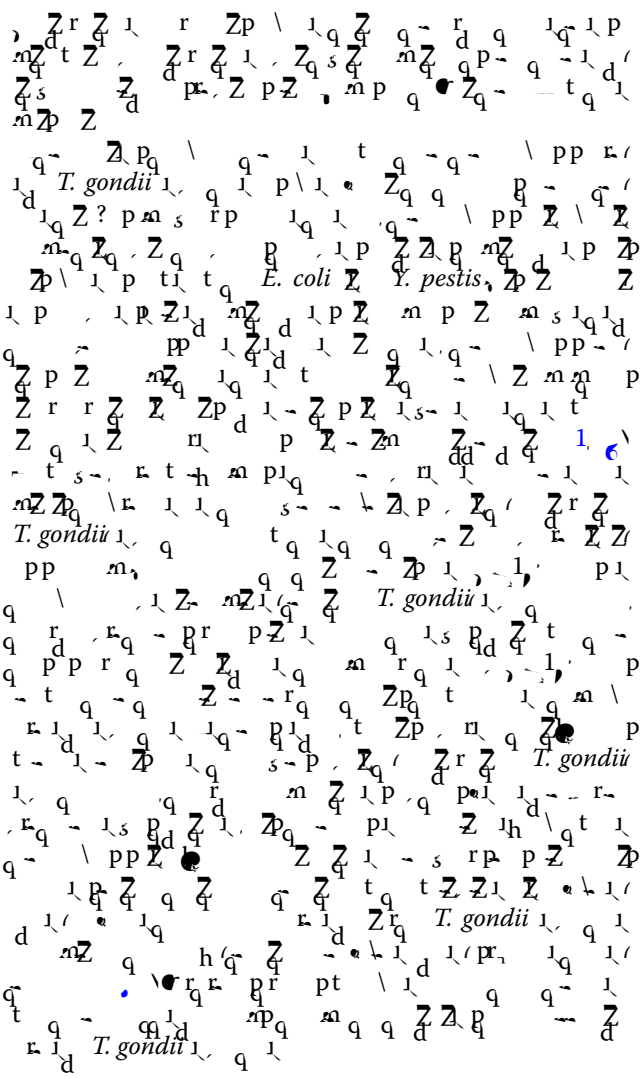


Fig. 7 Model of changes to the brain microvasculature during *T. gondii* infection. On the left is the brain microvasculature of an uninfected mouse, with no upregulation of adhesion molecules, and no changes to tortuosity. On the right, we demonstrate that in the brain microvasculature of *T. gondii*-infected mice there is increased tortuosity and adhesion molecules (VCAM-1 and ICAM-1), as well as increased coagulation, resulting in decreased cerebral blood flow (created using Biorender)



Conclusions

Toxoplasma gondii

Abbreviations

BBB	Blood-Brain Barrier
CBF	Cerebral Blood Flow
cIg	Control Ig
CNS	Central Nervous System
DPI	Days Post-Infection
FOV	Field Of View
i.p.	intraperitoneally
LSI	Laser Speckle Imaging
mAb	Monoclonal Antibody
rCBF	Relative Cerebral Blood Flow
ROI	Region Of Interest
TF	Tissue Factor
WT	Wild-Type

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12974-024-03330-1.

Supplementary Material 1

Supplementary Material 2

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Author contributions

E.M.H., C.A.S., C.C., T.S.L, D.X.F.V., S.P.G., B.C., and M.B.L. designed research; E.M.H., C.A.S., C.C., T.S.L, D.X.F.V., and C.J.T. performed research; D.A. contributed new reagents/analytic tools; E.M.H., C.A.S., C.C., T.S.L, and M.B.L. analyzed data; andE.M.H., C.A.S., C.C., T.S.L, D.X.F.V., C.J.T., D.A., S.P.G., B.C. and M.B.L. wrote the manuscript. All authors reviewed and approve of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All procedures and protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Irvine.

Consent for publication

All authors approved this manuscript and provided consent for publication.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Molecular Biology and Biochemistry, University of California Irvine, Irvine 92697, USA
[2]Institute for Immunology, University of California Irvine, Irvine 92697, USA
[3]Department of Biomedical Engineering, University of California Irvine, Irvine 92697, USA
[4]Beckman Laser Institute and Medical Clinic, University of California Irvine, Irvine 92697, USA
[5]Department of Biological Sciences, California State Polytechnic University, Pomona, CA 91768, USA
[6]Department of Neurobiology and Behavior, University of California Irvine, Irvine 92697, USA

[7]Center for the Neurobiology of Learning and Memory, University of California Irvine, Irvine 92697, USA
[8]Department of Surgery, University of California Irvine, Irvine 92697, USA
[9]Edwards Lifesciences Foundation Cardiovascular Innovation Research Center, University of California Irvine, Irvine 92697, USA
[10]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY 10032, USA
[11]Department of Neurology, Columbia University Irving Medical Center, New York, NY 10032, USA

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