Supplementary Information

C. difficile intoxicates neurons and pericytes to drive neurogenic inflammation

Supplementary Table 1: A list of antibody information.

Supplementary Figure 1: A figure containing the source western blot scans and Coomassie staining related to **Extended Data Figure 5f**.

Supplementary Video 1: Pericytes envelope colonic vascular network.
Mouse colonic tissue from Cspg4-DsRed mice was harvested, processed by whole mount, and subjected to immunostaining with BABB optical clearing of tissue. Z-stack images were captured on a Zeiss LSM 700 confocal microscope with Zen Black software. Z-stack images were processed and rendered into 3D image movies using Aivia image analysis software. In the 3D image movie, an initial view is seen peering down at epithelium lining bottom of intestinal crypts (background non-specific green fluorescence signal). Removal of epithelial background reveals CD31 immunolabeled capillary networks and a deeper arteriole (green). DsRed antibody immunolabeled Cspg4-DsRed reporter pericytes (magenta) are found in close juxtaposition to the vascular network throughout.

Supplementary Video 2: Pericytes wrap around mucosal capillaries.
Mouse colonic tissue from Cspg4-DsRed mice was harvested, processed by cryosection, and subjected to immunostaining. Z-stack images were captured on a Zeiss LSM 880 confocal microscope with Zen Black software. Z-stack images were processed and rendered into 3D image movies using Aivia image analysis software. The movie demonstrates a 3D-view of two CSPG4-reporter labeled pericytes (magenta). CD31 immunolabeled labeled capillaries (green) are found nestled within the pericyte bodies and wrapped within pericyte cellular processes.

Supplementary Video 3: Colonic neurovascular unit comprised of pericyte, capillary, CGRP+ neuron processes.
Mouse colonic tissue from Cspg4-DsRed mice was harvested, processed by cryosection, and subjected to immunostaining. Z-stack images were captured on a Zeiss LSM 880 confocal microscope with Zen Black software. Z-stack images were processed and rendered into 3D image movies using Aivia image analysis software. The movie demonstrates a 3D-image of a colonic neurovascular unit. A single CSPG4-dsRed reporter labeled pericyte (magenta) is shown. A CD31 immunolabeled vessel (green) is surrounded by the pericyte body and its processes. CGRP immunolabeled neuron processes (gray) appear to interact with the pericyte at multiple points.

Supplementary Table 1

Primary Antibodies

Target	Host	Vendor	Cat. No.	Concentration
Calcrl	Rabbit	Sigma Aldrich	HPA008070	1:1000
CD31	Goat	R&D Systems	AF3628	1:1000
CD31	Rat	BD Biosciences	553708	1:500
CGRP	Rabbit	Immunostar	24112	1:1000-2000
DsRed	Rabbit	TakaRa	632496	1:500-1000
GFP	Chicken	Aves	GFP-1020	1:1000
GFP	Rabbit	Invitrogen	A11122	1:1000
HuC/D	Rabbit	Abcam	ab184267	1:1000-2000
HuC/D (ANNA1)	Human	gift V. Lennon	n/a	1:1000-2000
Lyve1	Goat	R&D Systems	AF2125	1:1000
Pdgfrα	Goat	R&D Systems	AF1062	1:1000
Ramp1	Rabbit	Alomone	ARR-021	1:1000
5HT (serotonin)	Goat	Immunostar	20079	1:1000
αSmooth Muscle Actin	Rabbit	Abcam	ab5694	1:1000
Smooth Muscle Actin (1A4)	Mouse	Sigma Aldrich	A5228	1:200
CD117/cKit	Goat	R&D Systems	AF1356	1:1000
Substance P	Rabbit	Immunostar	20064	1:100-500
Tubb3 (Tuj1)	Mouse	Biolegend	801201	1:500-2000

Secondary Antibodies

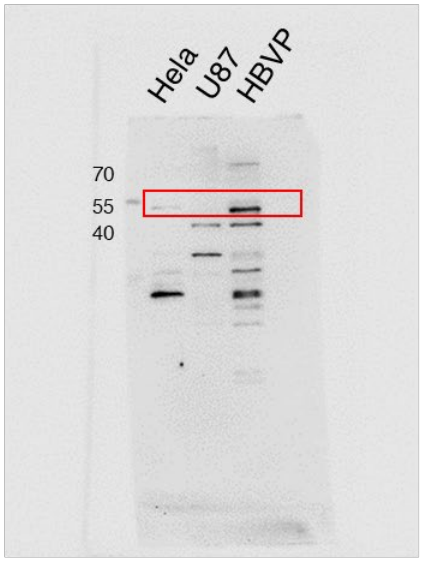
Secondary antibodies for immunohistochemistry were all applied at a concentration of 1:500 unless otherwise specified.

Antibody	Fluorophore	Vendor	Cat. No.
Donkey anti-Rabbit IgG (H+L)	Alexa Fluor 594	Life Tech/Invitrogen	A21207
Donkey anti-Rabbit IgG (H+L)	Alexa Fluor 647	Invitrogen	A32795
Donkey anti-Rabbit IgG (H+L)	Alexa Fluor 488	Life Tech/Invitrogen	A21206

Donkey anti-Rabbit IgG (H+L)	Alexa Fluor 488	Abcam	ab150073
Donkey anti-Rat IgG (H+L)	Alexa Fluor 488	Invitrogen	A21208
Donkey anti-Goat IgG (H+L)	Alexa Fluor 568	Invitrogen	A11057
Donkey anti-Goat IgG (H+L)	Alexa Fluor 647	Life Tech/Invitrogen	A21447
Donkey anti-Goat IgG	CF 647	Millipore	SAB4600175
Donkey anti-Goat IgG (H+L)	Alexa Fluor 488	Invitrogen	A11055
Donkey anti-Goat IgG (H+L)	Alexa Fluro 647	Invitrogen	A21447
Donkey anti-Chicken IgY (H+L)	Alexa Fluor 488	Invitrogen	A78948
Donkey anti-Chicken IgY (H+L)	FITC	Invitrogen	SA1-72000
Donkey anti-Human IgG (H+L)	Alexa Fluor 647	Jackson	709-605-149
Donkey anti-Human IgG (H+L)	DyLight 594	Thermofisher	SA5-100215
Donkey anti-Mouse IgG (H+L)	Alexa Fluor 647	Jackson	715-605-151
Donkey anti-Mouse IgG (H+L)	Alexa Fluor 647	Invitrogen	A31571
Donkey anti-Mouse IgG (H+L)	Alexa Fluor488	Abcam	ab150109
Goat anti-Rabbit IgG (H+L)	Alexa Fluor 647	Life Tech/Invitrogen	A21244
Goat anti-Rat IgG (H+L)	Alexa Fluor 647	Life Tech/Invitrogen	A21247
Goat anti-Rat IgG (H+L)	Alexa Fluor 488	Invitrogen	A11006
Goat anti-Mouse IgG2a	Alexa Fluor 488	Life Tech/Invitrogen	A21131
Goat anti-Chicken IgY (H+L)	Alexa Fluor 488	Invitrogen	A11039
Goat anti-Rabbit IgG (H+L)	Alexa Fluor 488	Jackson	111-545-144
Goat anti-Mouse IgG (H+L)	Alexa Fluor 647	Invitrogen	A-21236

Supplementary Figure 1.

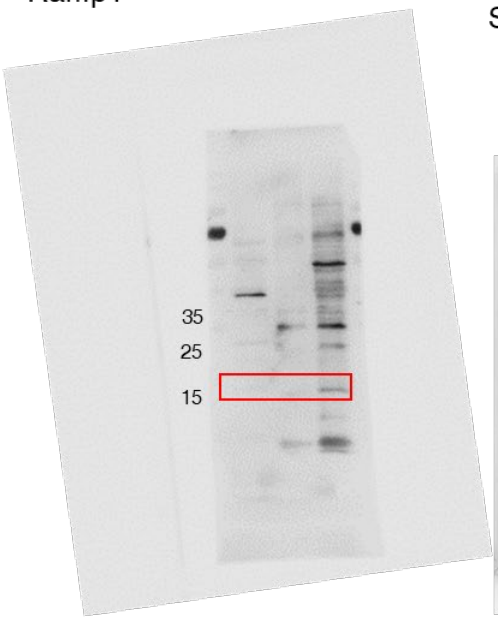
Calcrl



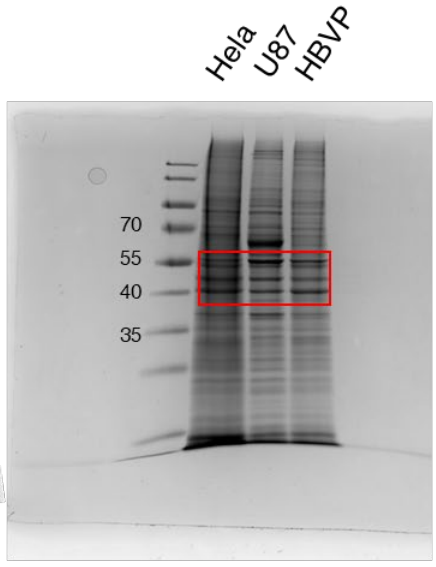
Sigma-Aldrich Antibody

Additional Validation
Not shown
BIOSS Antibody

Ramp1



Sample Processing Control - Coomassie



Supplementary Figure 1: Full Western blot scans related to **Ext. Data Figure 5l,** and full scan of Cooomassie staining of SDS-PAGE (sample processing control).