

Supplementary Figures

Sephin1 preserves visual circuit function and retinogeniculate integrity during autoimmune inflammatory demyelination

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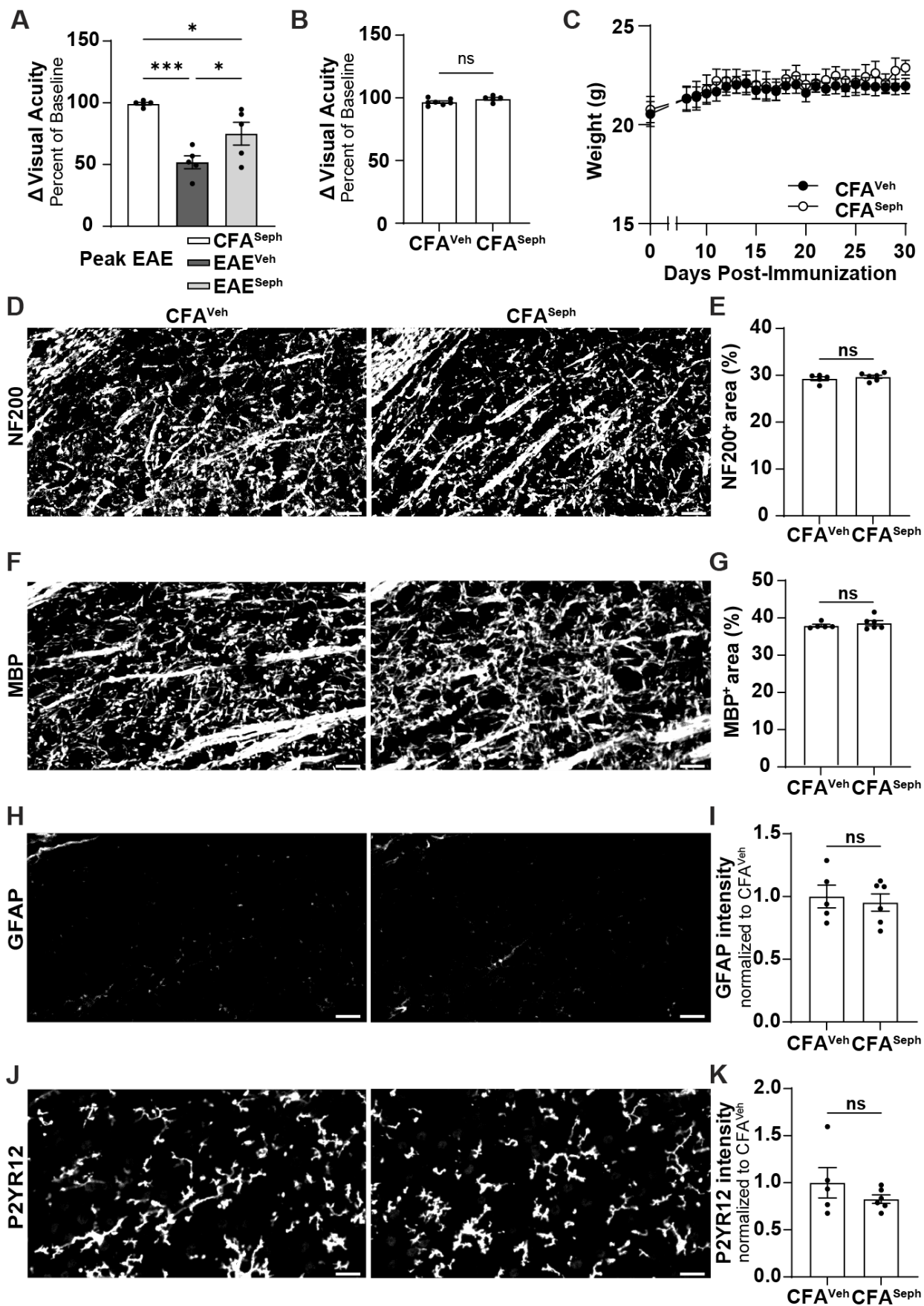


Figure S1: Sephin1 does not alter visual acuity and hallmark histological features in CFA^{Seph} controls. (A) Quantification of visual acuity (cycles/degree) as a percent of baseline values at peak EAE (16 dpi) shows a protection of visual function for EAE^{Seph} compared to EAE^{Veh} mice. (B) Quantification of visual acuity between CFA controls treated with vehicle or Sephin1 shows no change relative to their baseline values. (C) Weights for CFA^{Veh} and CFA^{Seph} controls across 30 days are similar. (D,F,H,J) All histological images were taken within the LGN. (D) Representative immunostaining against NF200⁺ axons. (E) Quantification of NF200⁺ percent area shows no difference between CFA^{Veh} and CFA^{Seph} controls. (F) Representative immunostaining against MBP⁺ myelin. (G) Quantification of MBP⁺ percent area shows no difference between CFA^{Veh} and CFA^{Seph} controls. (H) Representative immunostaining against GFAP⁺ astrocytes. (I) Quantification of GFAP⁺ signal intensity shows no difference between CFA^{Veh} and CFA^{Seph} controls. (J) Representative immunostaining against P2RY12⁺ microglia. (K) Quantification of P2RY12⁺ signal intensity shows no difference between CFA^{Veh} and CFA^{Seph} controls. All scale bars=20µm. Data=mean±SEM, n=5-6/group Statistical analyses: one-way ANOVA with Tukey's post-hoc test (A) or unpaired t test (B, E, G, I, K). ns=not significant, (A) CFA^{Seph} vs EAE^{Veh} $P=0.0004$; CFA vs EAE^{Seph} $P=0.0397$; EAE^{Veh} vs EAE^{Seph} $P=0.0489$, (B) $P=0.1053$; (E) $P=0.4756$, (G) $P=0.4308$, (I) $P=0.6780$, (K) $P=0.2862$.

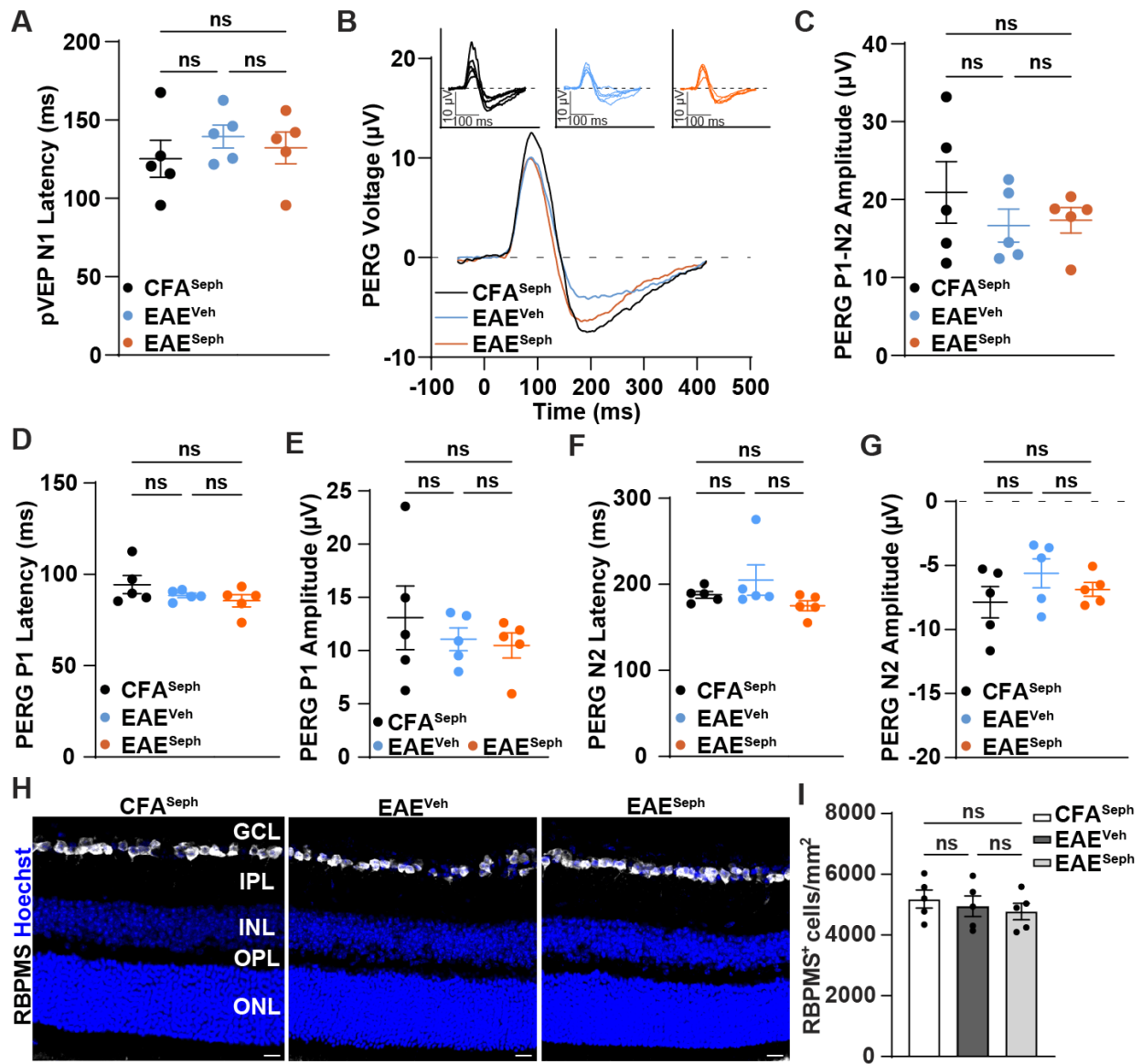


Figure S2: pVEP latency, PERG responses, and RGC numbers are unaltered at peak EAE.

(A) Quantification of pVEP N1 latency at peak EAE shows no change across experimental groups.

(B) Average PERG traces at peak EAE across experimental groups. Insets show individual traces for each animal per group.

Quantification of: **(C)** PERG P1-N2 amplitude, **(D)** PERG P1 latency,

(E) PERG P1 amplitude, **(F)** PERG N2 latency, **(G)** PERG N2 amplitude.

(H) Representative immunostaining against retinal ganglion cell (RGC) marker RBPMS (white) and Hoechst nuclear

indicator (blue). All other layers of the retina are qualitatively similar across groups including inner

Figure S2 (continued):

plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), and outer nuclear layer (ONL). Scale bars=20µm. **(I)** Quantification of RBPMS⁺ cells in the RGC layer of retinal cross-sections. No statistical differences were observed across any quantifications. Data=mean±SEM, n=5/group Statistical analyses: one-way ANOVA with Tukey's post-hoc test (A, C-G, I). ns=not significant, (A) CFA^{Seph} vs EAE^{Veh} $P=0.5865$; CFA vs EAE^{Seph} $P=0.8735$; EAE^{Veh} vs EAE^{Seph} $P=0.8684$, (C) CFA^{Seph} vs EAE^{Veh} $P=0.5332$; CFA vs EAE^{Seph} $P=0.6372$; EAE^{Veh} vs EAE^{Seph} $P=0.9831$, (D) CFA^{Seph} vs EAE^{Veh} $P=0.4807$; CFA vs EAE^{Seph} $P=0.2206$; EAE^{Veh} vs EAE^{Seph} $P=0.8330$, (E) CFA^{Seph} vs EAE^{Veh} $P=0.7501$; CFA vs EAE^{Seph} $P=0.6256$; EAE^{Veh} vs EAE^{Seph} $P=0.9760$, (F) CFA^{Seph} vs EAE^{Veh} $P=0.5269$; CFA vs EAE^{Seph} $P=0.7008$; EAE^{Veh} vs EAE^{Seph} $P=0.1742$, (G) CFA^{Seph} vs EAE^{Veh} $P=0.2913$; CFA vs EAE^{Seph} $P=0.7707$; EAE^{Veh} vs EAE^{Seph} $P=0.6597$, (I) CFA^{Seph} vs EAE^{Veh} $P=0.8488$; CFA vs EAE^{Seph} $P=0.6144$; EAE^{Veh} vs EAE^{Seph} $P=0.9117$.

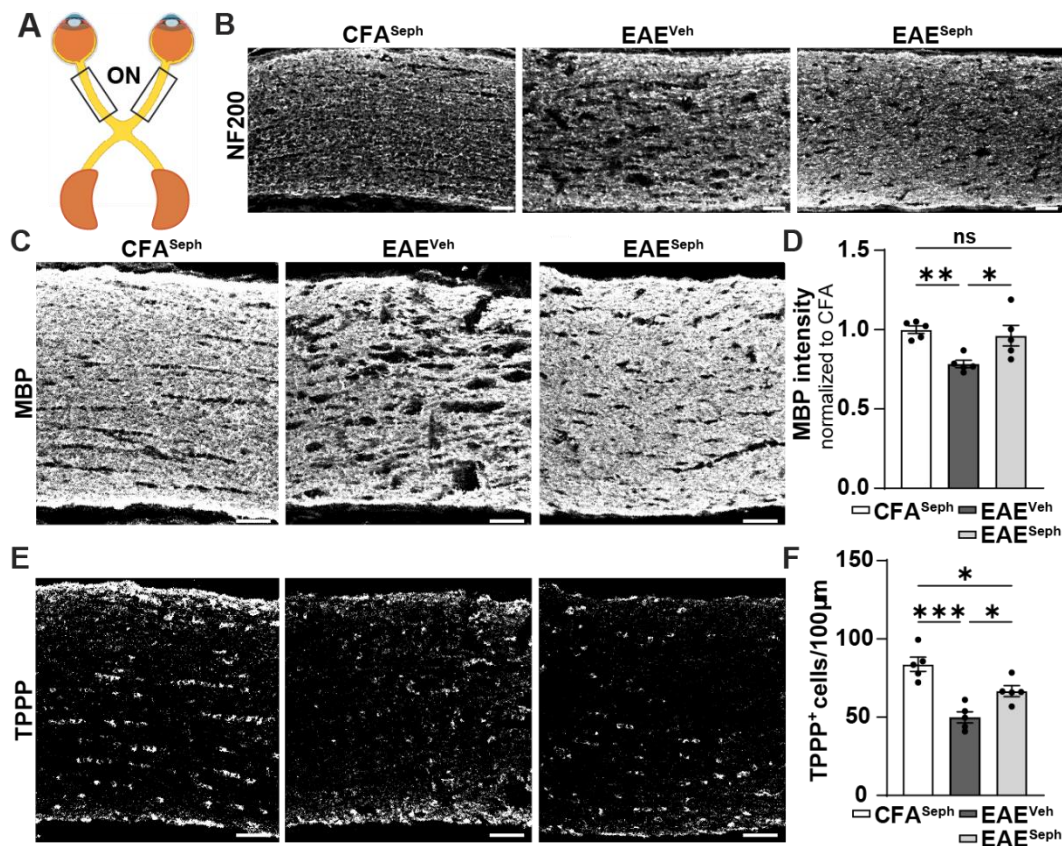


Figure S3: Sepsin1 preserves optic nerve myelin and oligodendrocytes. (A) Schematic representation of the retinogeniculate pathway with boxes around the region of interest, the optic nerve (ON). (B) Representative immunostaining against NF200⁺ axons in 10x optic nerve images. (C) Representative immunostaining against MBP⁺ myelin. (D) Quantification of MBP⁺ intensity shows an increase in EAE^{Seph} compared to EAE^{Veh} mice. (E) Representative immunostaining against TPPP⁺ oligodendrocytes. (F) Quantification of TPPP⁺ cells per 100 microns shows that both EAE^{Veh} and EAE^{Seph} mice have a significant decrease relative to CFA^{Seph} controls, but TPPP⁺ cells are increased in EAE^{Seph} compared to EAE^{Veh} mice. All scale bars=50μm. Data=mean±SEM, n=5/group. Statistical analyses: one-way ANOVA with Tukey's post-hoc test (D, F). ns=not significant, (D) CFA^{Seph} vs EAE^{Veh} $P=0.0091$; CFA vs EAE^{Seph} $P=0.8066$; EAE^{Veh} vs EAE^{Seph} $P=0.0282$, (F) CFA^{Seph} vs EAE^{Veh} $P=0.0001$; CFA vs EAE^{Seph} $P=0.0234$; EAE^{Veh} vs EAE^{Seph} $P=0.0252$.

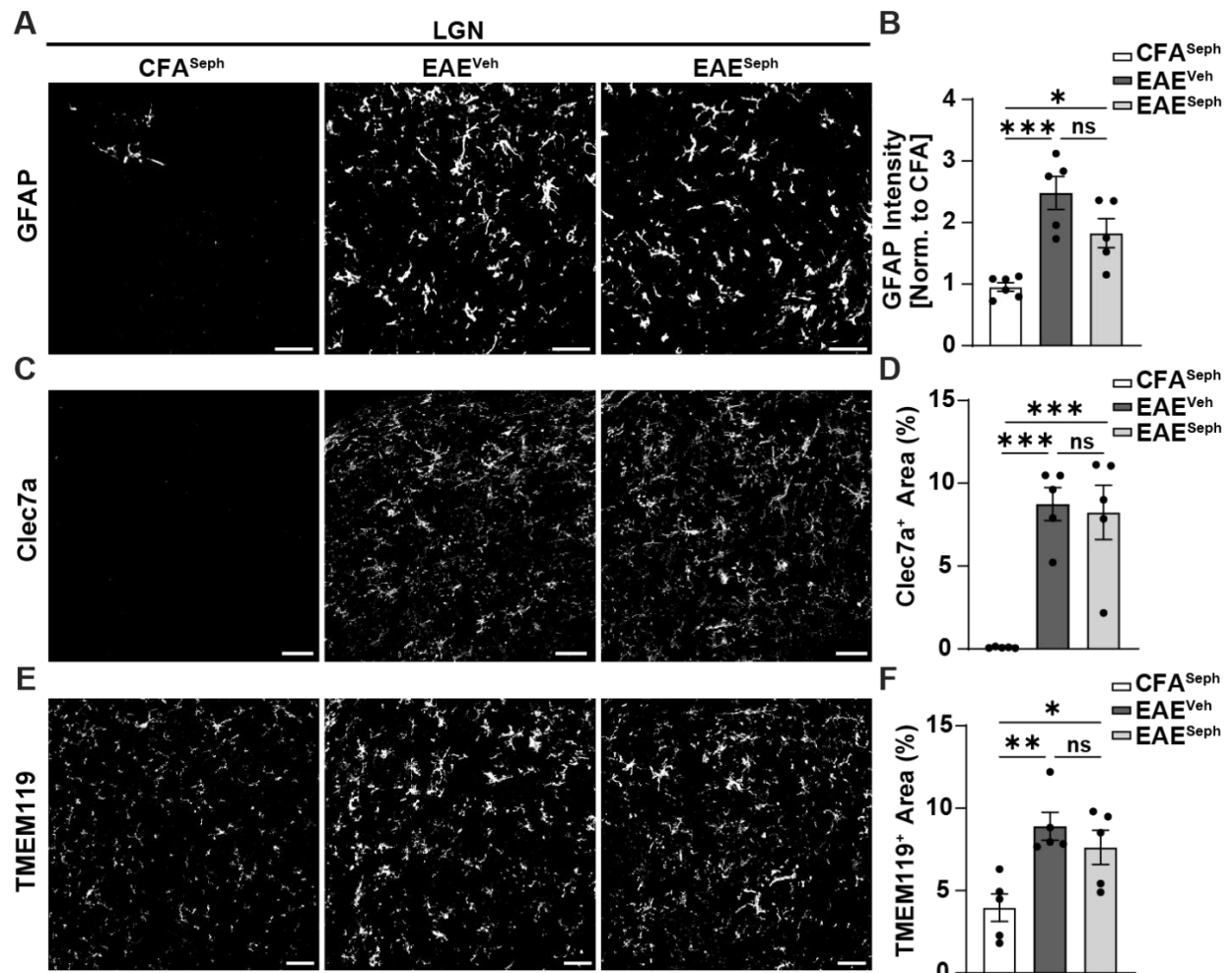


Figure S4: Sepsin1 does not protect against reactive gliosis in the LGN at peak EAE. (A) Representative immunostaining against GFAP⁺ astrocytes. **(B)** Quantification of GFAP⁺ signal intensity normalized to CFA^{Seph} controls shows a significant increase in both EAE^{Veh} and EAE^{Seph} compared to CFA^{Seph} controls, with no significant differences between the EAE groups. **(C)** Representative immunostaining against Clec7a⁺ microglia. **(D)** Quantification of Clec7a⁺ percent area shows a significant increase in both EAE^{Veh} and EAE^{Seph} mice compared to CFA^{Seph} controls, with no significant differences between EAE groups. **(E)** Representative immunostaining against TMEM119⁺ microglia. **(F)** Quantification of TMEM119⁺ percent area shows a significant increase in both EAE^{Veh} and EAE^{Seph} compared to CFA^{Seph} controls, with no significant differences between the EAE groups. All scale bars=40μm. Data=mean±SEM, n=5/group. Statistical analyses: one-

Figure S4 (continued):

way ANOVA with Tukey's post-hoc test (B, D, F). ns=not significant, (B) CFA^{Seph} vs EAE^{Veh} $P=0.0003$; CFA^{Seph} vs EAE^{Seph} $P=0.0186$; EAE^{Veh} vs EAE^{Seph} $P=0.0967$, (D) CFA^{Seph} vs EAE^{Veh} $P=0.0004$; CFA^{Seph} vs EAE^{Seph} $P=0.0006$; EAE^{Veh} vs EAE^{Seph} $P=0.9463$, (F) CFA^{Seph} vs EAE^{Veh} $P=0.0063$; CFA^{Seph} vs EAE^{Seph} $P=0.0369$; EAE^{Veh} vs EAE^{Seph} $P=0.5993$.

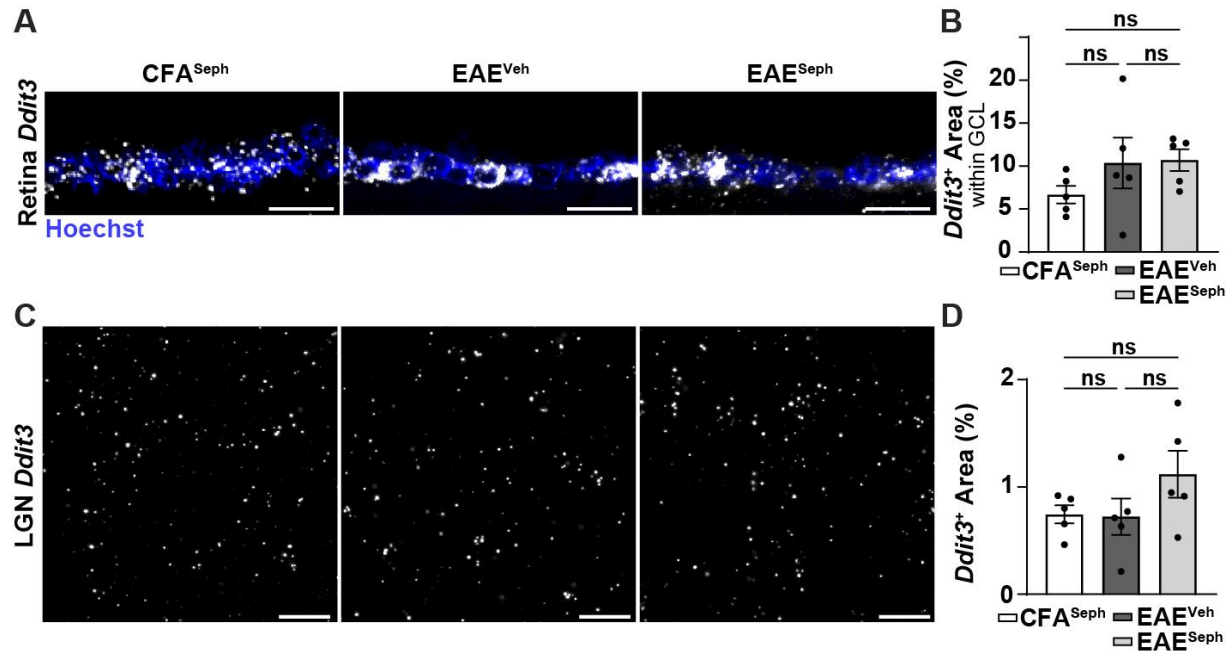


Figure S5: *Ddit3* is not altered in the retina or LGN at peak EAE. (A) Representative *Ddit3* mRNA in the GCL of the retina. (B) Quantification *Ddit3*⁺ percent area in the GCL shows no significant differences across any experimental group. (C) Representative *Ddit3* mRNA in the LGN. (D) Quantification of *Ddit3*⁺ percent area shows no significant differences across any experimental group. All scale bars=20μm. Data=mean±SEM, n=5/group. Statistical analyses: one-way ANOVA with Tukey's post-hoc test (B, D). ns=not significant, (B) CFA^{Seph} vs EAE^{Veh} $P=0.4023$; CFA^{Seph} vs EAE^{Seph} $P=0.3430$; EAE^{Veh} vs EAE^{Seph} $P=0.9917$, (D) CFA^{Seph} vs EAE^{Veh} $P=0.9947$; CFA^{Seph} vs EAE^{Seph} $P=0.2886$; EAE^{Veh} vs EAE^{Seph} $P=0.2508$.