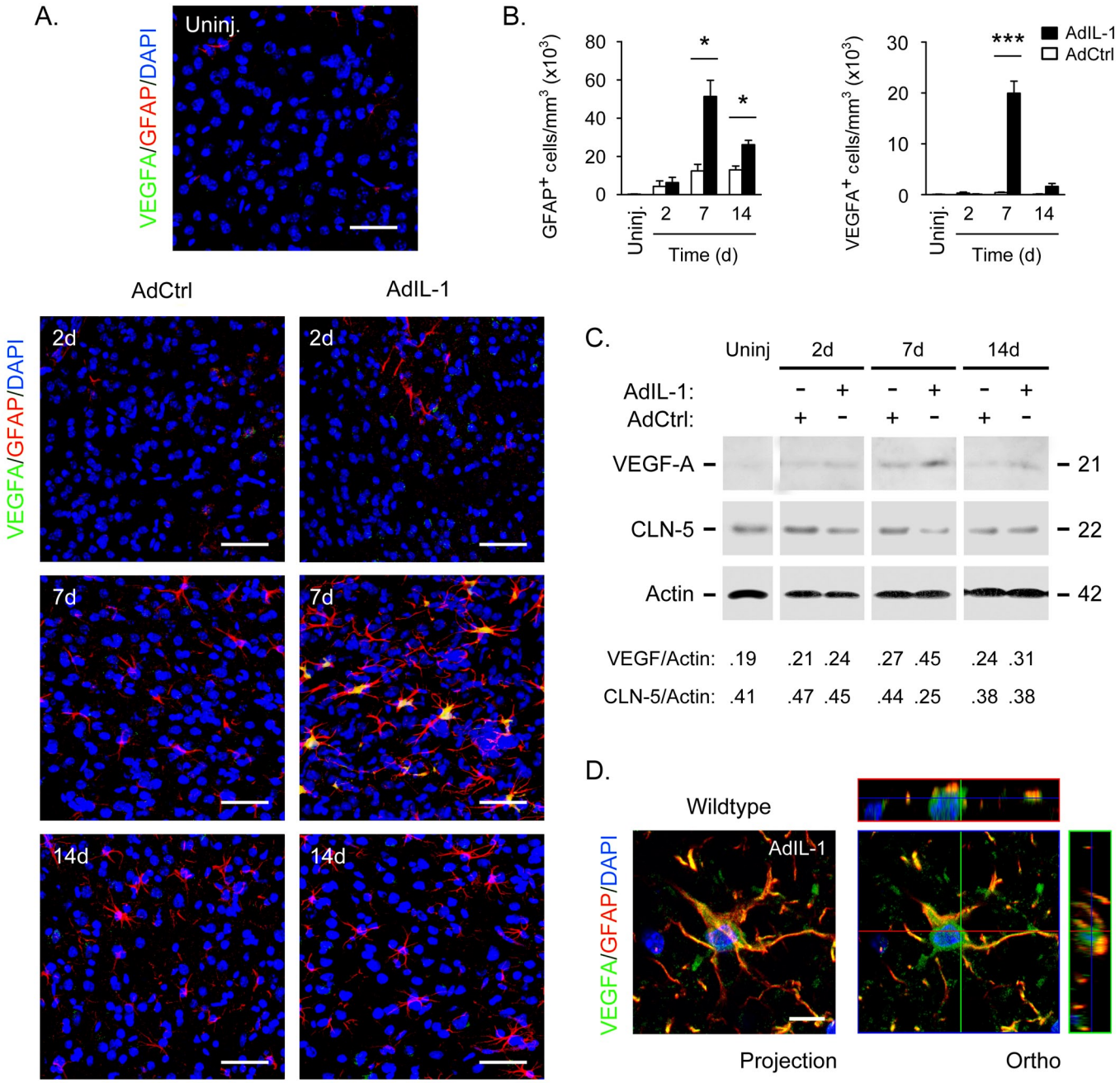
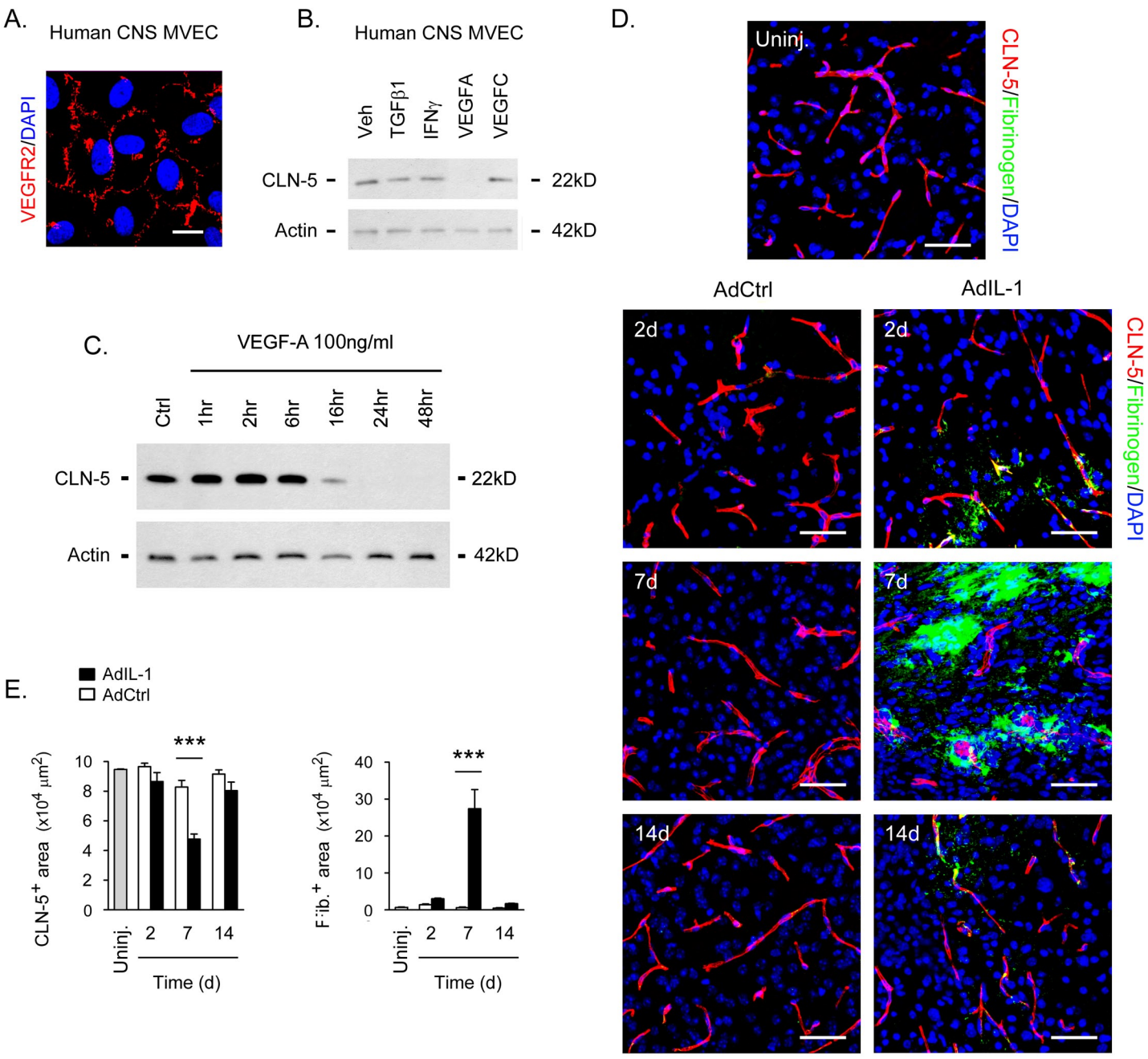
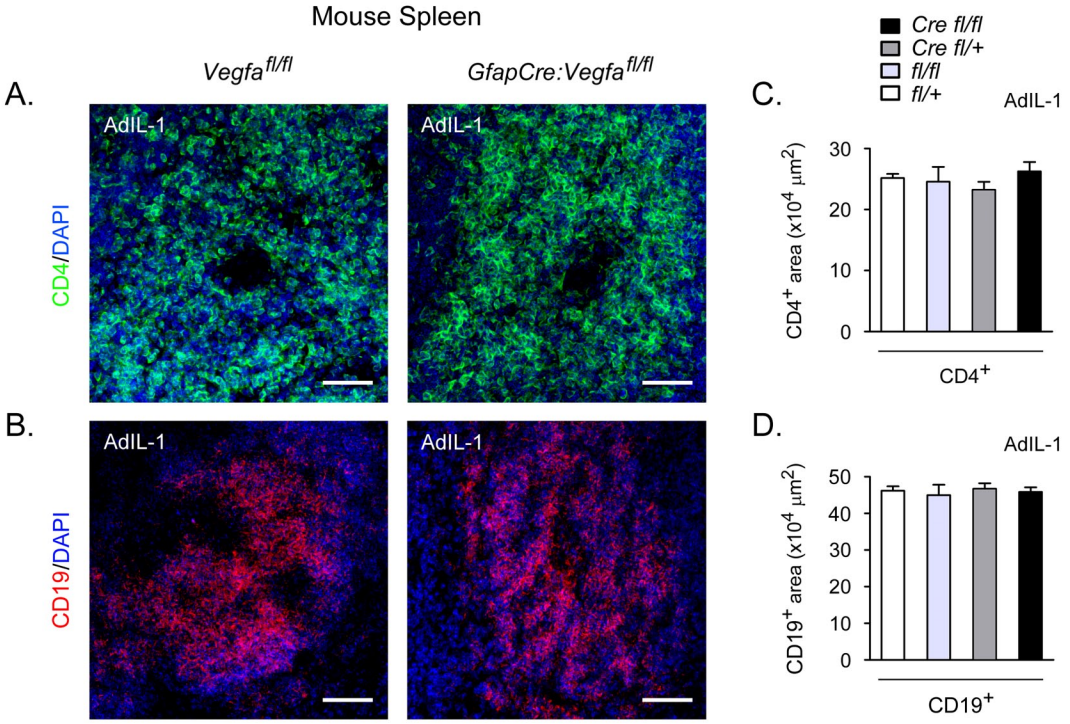


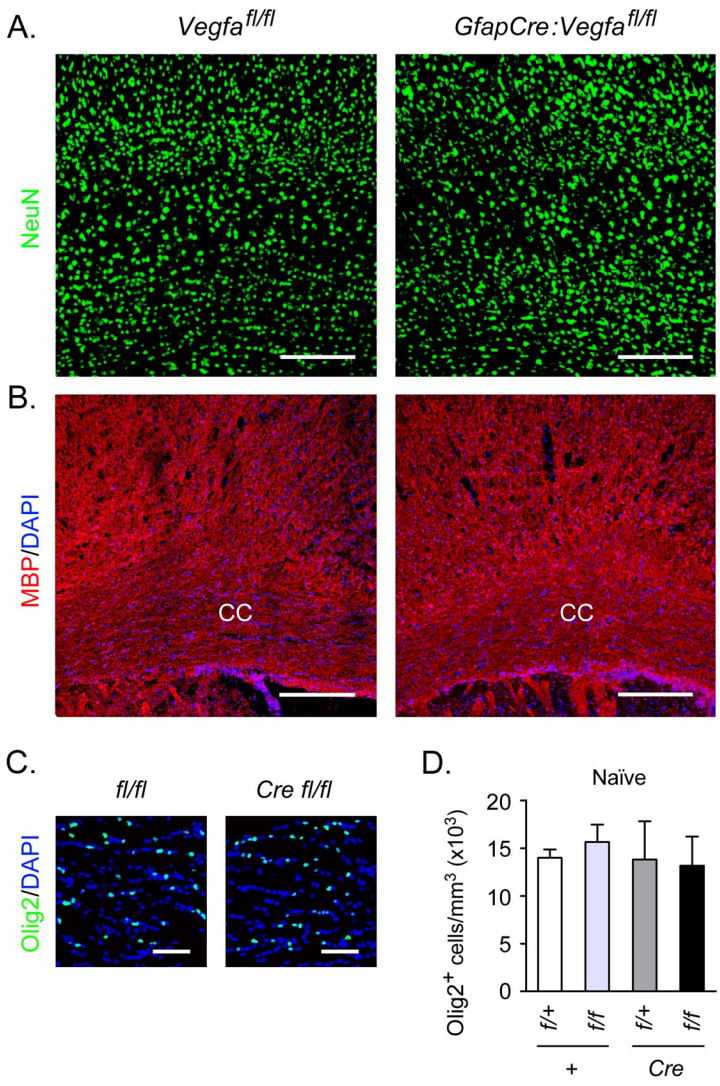
Supplementary Figure 1. Timecourse of VEGF-A induction in AdIL-1 lesions in adult mice. (A-C) C57BL/6 mice (12wk, 6 per condition per timepoint) received cortical microinjection of AdIL-1 or AdCtrl (10^7 pfu), and were sacrificed at 2, 7 or 14dpi. AdIL-1-injected cortices contained hypercellular lesions in which VEGF-A localized to $GFAP^+$ reactive astrocytes **(A)**. Numbers of $VEGF\text{-}A^+GFAP^+$ cells were maximal at 7dpi **(B)**. **(C)** Immunoblotting confirmed induction of VEGF-A in AdIL-1-injected cortices at 7dpi. This effect coincided with downregulation of CLN-5. Data shown are from noncontiguous lanes of the same gel. In **(D)**, a high magnification confocal projection (left panel) and matching ortho-image (right panel) from AdIL-1-injected cortex show localization of VEGF-A to a $GFAP^+$ cell (left panel), and illustrate its pattern of immunoreactivity, distinct from GFAP (right panel). Scale, **(A)** 50μm, **(D)** 5μm. Statistics, **(B,C)** ANOVA plus Bonferroni test, *P<0.05, ***P<0.001. Data are representative of 3 independent experiments.



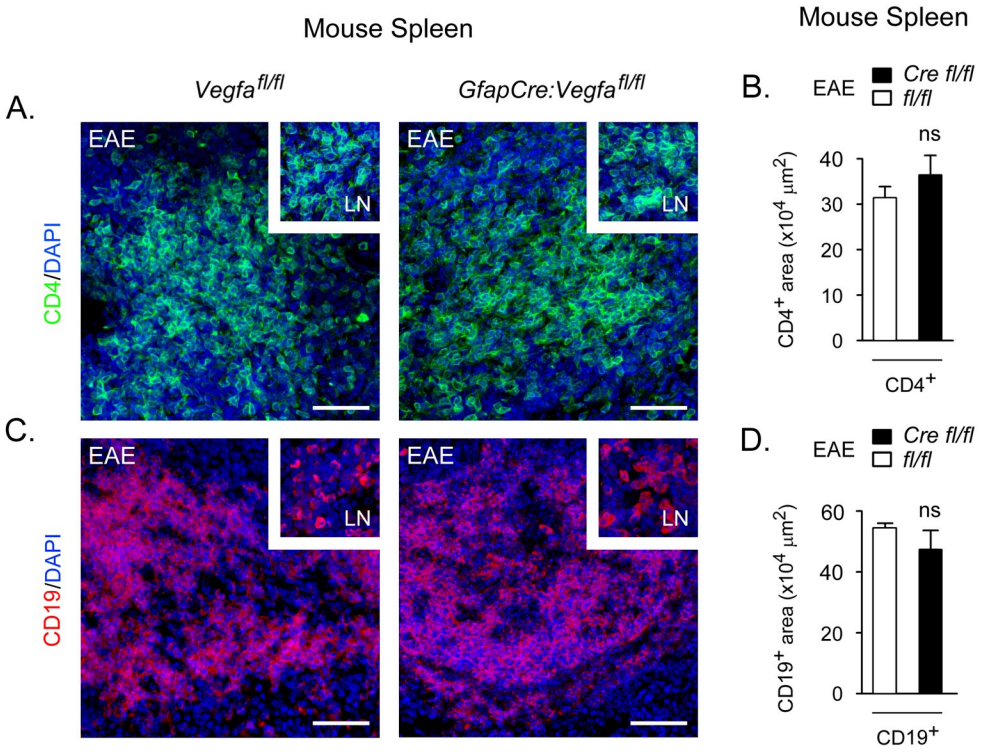
Supplementary Figure 2. Timecourse of CLN-5 disruption in CNS endothelium in vitro and in vivo. (A) Immunostaining of human CNS MVECs shows localization of VEGFR2 immunoreactivity to the cell membrane. **(B,C)** In immunoblotting studies on human CNS MVECs, CLN-5 was downregulated at 24h in VEGF-A-treated cultures (10ng/ml), but did not respond to other factors tested **(B)**. Following VEGF-A treatment, CLN-5 was downregulated at 16h and undetectable at 24 and 48h **(C)**. **(D,E)** C57BL/6 mice (12wk, 3 per condition per timepoint) received cortical microinjection of AdIL-1 or AdCtrl (10^7 pfu), then were sacrificed at 2, 7 or 14dpi as in **Suppl.Fig.1**. In uninjected mice, cortical expression of CLN-5 was robust, and no parenchymal leakage of serum proteins was detected **(D)**. AdIL-1-injected cortices exhibited disruption of CLN-5 immunoreactivity, and BBB breakdown as measured by extravasation of fibrinogen **(D)**. These effects were maximal at 7dpi (**E**, see also **Suppl.Fig.1C**). Empty vector control had no detectable effect **(D,E)**. Scale **(A)**, 10μm, **(D)** 50μm. Statistics, **(E)** ANOVA plus Bonferroni test, ***P<0.001. Data in **(A-C)** are representative of studies in 3 independent cultures. Panels **(D,E)** are representative of 3 independent experiments.



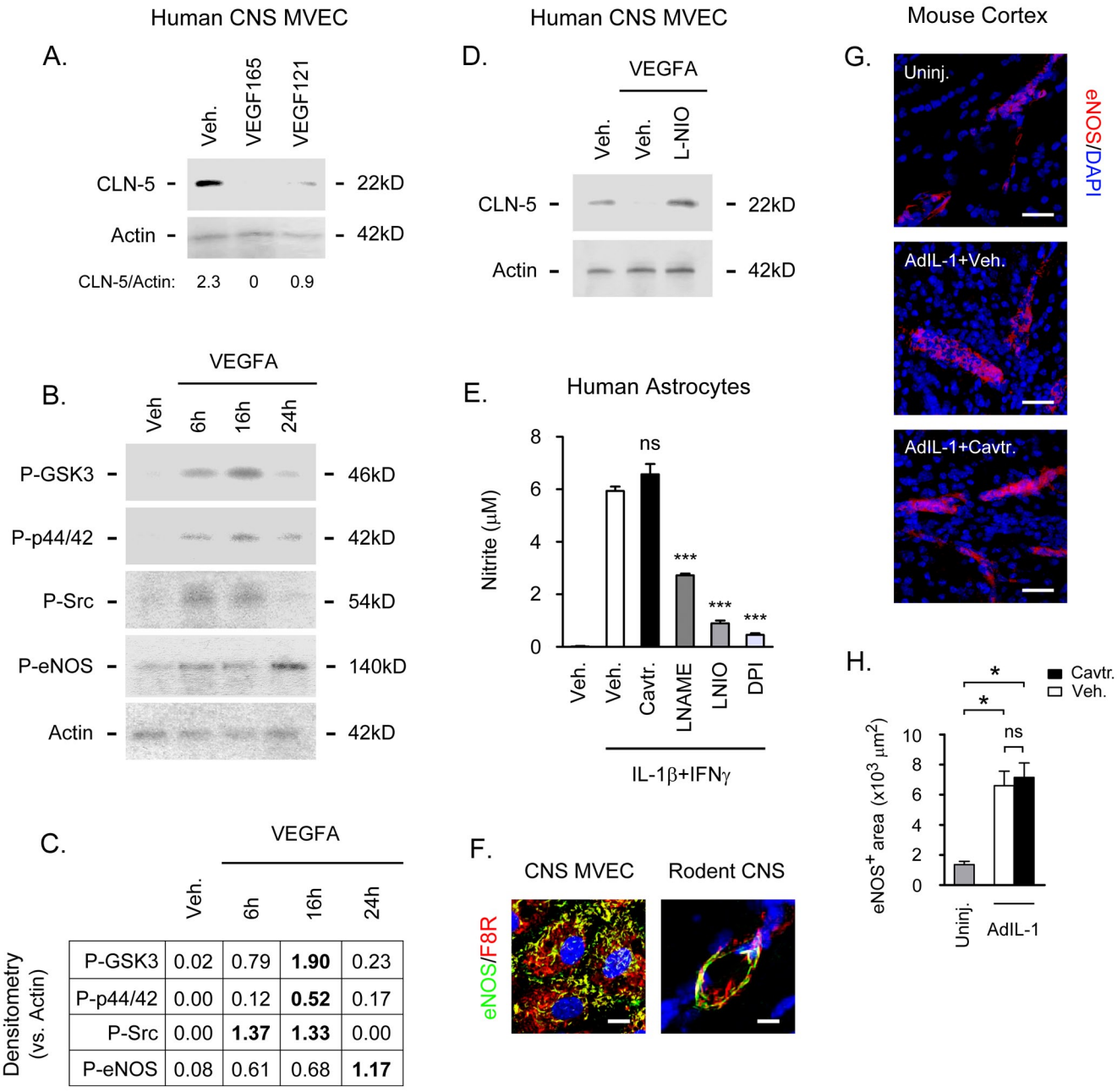
Supplementary Figure 3. Peripheral lymphoid organs of AdIL-1-injected *GfapCre:Vegfa*$^{fl/fl}$ mice and controls are indistinguishable. Spleens from 12wk *GfapCre:Vegfa*$^{fl/fl}$ mice and littermates (7dpi, n=3 per genotype) subjected to CNS microinjection of 10^7pfu AdIL-1 and sacrificed at 7dpi, immunostained for CD4 and CD19. Sections from spleens of *GfapCre:Vegfa*$^{fl/fl}$ mice and littermates of other genotypes were indistinguishable **(A,B)**, and the areas of $CD4^+$ and $CD19^+$ immunoreactivity were the same in all four genotypes **(C,D)**. Scalebars **(A,B)** 50µm. Statistics **(C,D)**, ANOVA. Data are typical of 3 independent experiments.



Supplementary Figure 4. Neuroanatomy of cerebral cortices is normal in *GfapCre:Vegfa*$^{fl/fl}$ mice. Cortical sections of naïve 12wk *GfapCre:Vegfa*$^{fl/fl}$ mice and littermates (12wk, n=3 per genotype) were stained for NeuN **(A)**, MBP **(B)**, and Olig2 **(C,D)**. Panel **(A)** shows cortical grey matter, while the corpus callosum (CC) is marked in panel **(B)**. **(A)** Numbers and organization of cortical NeuN^{+} neurons were normal in unchallenged *GfapCre:Vegfa*$^{fl/fl}$ adults. Immunoreactivity for the myelin protein MBP **(B)** and numbers of Olig2^{+} oligodendrocytes **(C,D)** were identical in all genotypes. Scale, **(A,B)** 150μm, **(C)** 15μm. Statistics, **(D)** ANOVA.



Supplementary Figure 5. No differences in peripheral lymphoid organs of *GfapCre:Vegfa*$^{fl/fl}$ mice and controls with EAE. Spleens (main panels) and lymph nodes (inset) from 8wk *GfapCre:Vegfa*$^{fl/fl}$ mice and *Vegfa*$^{fl/fl}$ littermates sensitized with MOG_{35-55} in CFA (21dpi, n=4 per genotype). No differences were detected in immunoreactivity for CD4 **(A,B)** or CD19 **(C,D)**. Scalebars **(A,C)** 50µm. Statistics **(B,D)**, ANOVA.



Supplementary Figure 6. Activation of VEGF-A signaling in CNS endothelium. **(A)** CNS MVECs were treated for 24h with $VEGF_{121}$ (binds to VEGFR2 but not its coreceptor NRP1) or $VEGF_{165}$ (a ligand for both). The effect of $VEGF_{121}$ on CLN-5 expression was slightly weaker than $VEGF_{165}$. **(B,C)** CNS MVECs were treated with 10ng/ml VEGF-A for times shown. VEGF-A induced phosphorylation of the PI3kinase pathway transcription factor GSK3(S21/9) and MEK kinase p44/42(T202/Y204), with responses peaking at 16h. VEGF-A also induced Src(Y416) phosphorylation, which was prominent at 6h and 16h. In contrast, VEGF-induced phosphorylation of eNOS(S1177) increased progressively to 24h. **(D)** CNS MVECs were exposed to the NOS inhibitor L-NIO (100μM) or vehicle 2h, then treated with 10ng/ml VEGF-A for 24h. L-NIO inhibited VEGF-A downregulation of CLN-5. **(E)** Primary human astrocytes were pretreated 2h with cavtratin (2μM) or the NOS inhibitors L-NAME, L-NIO (both 100μM) or DPI (50μM) for 2h, then exposed to 10ng/ml IL-1β+IFNγ to activate iNOS expression and nitrite production, which was measured in supernatant at 24h. Cavtratin had no impact on nitric oxide production in this system. All other inhibitors tested significantly reduced nitrite levels. **(F)** Immunostaining of human CNS MVEC cultures (left) or adult mouse cortex (right) showed that eNOS immunoreactivity localized to endothelium. **(G,H)** 12wk C57BL/6 mice (3 per group) receiving cortical AdIL-1 (10^7 pfu) were given 2.5mg/kg cavtratin or vehicle i.p 30min pre-injection, then daily post-injection for 7d as in **Figs.7L,M**. Endothelial eNOS expression was increased in AdIL-1 lesions. Consistent with its post-translational mechanism of action, cavtratin did not alter eNOS immunoreactivity. Scale **(F)**, both panels 5μm, **(G)** 10μm. Statistics, **(H)** ANOVA plus Bonferroni test, *P<0.05. Data in **(A-F)** are typical of 3 separate cultures of human CNS MVECs **(A-D,F)** or astrocytes **(E)**. Data in **(G,H)** are representative of 3 independent experiments.