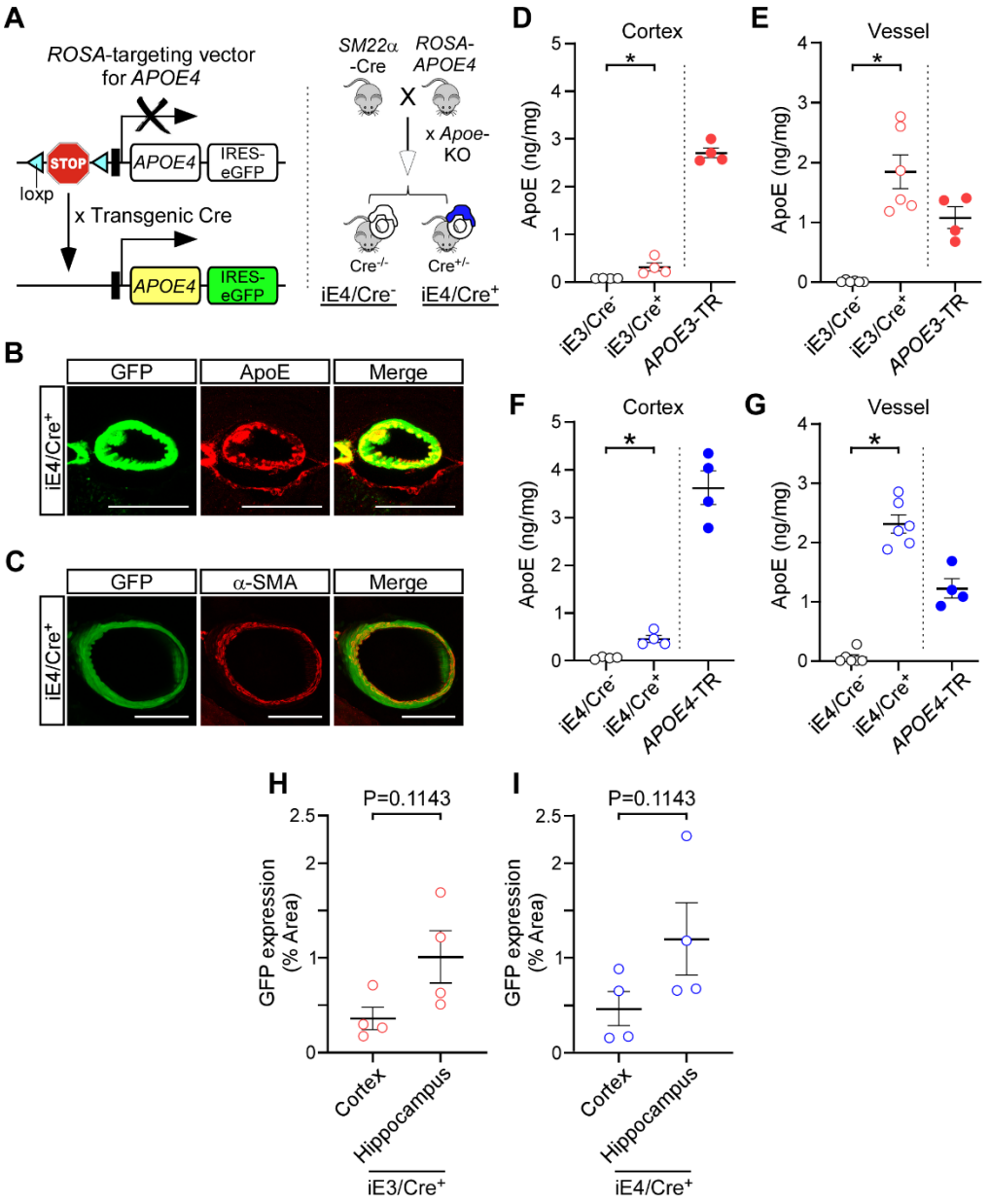

Figure S1. Generation and characterization of mouse models conditionally express human apoE isoforms in VMCs, Related to Figure 1.

(A) Strategy for generating cell-type specific *APOE* conditional mouse models. *Left,* illustration of *APOE4-eGFP* construct containing a floxed *STOP* cassette integrated into the *ROSA-26* locus. Breeding with *SM22alpha*-driven Cre recombinase mice allows apoE4 expression in VMCs. *Right,* upon further breeding with *Apoe*-KO mice, the resultant mice were named iE4/Cre^{+} mice. Unless otherwise stated, phenotypes driven by VMC-derived apoE4 were determined by comparing iE4/Cre^{+} mice with their Cre-negative littermates (iE4/Cre^{-}).

(B and C) Brain sections from iE4/Cre^{+} mice were immunolabeled for apoE (B), and smooth muscle cell marker (C, α-smooth muscle actin, α-SMA). Representative images are shown (n=3, all males). Scale bar, 50 μm (B), 100 μm (C).

(D and E) The amount of apoE in cortical tissues (D), and isolated brain vessels (E) was determined by ELISA in iE3/Cre^{-} (n=4-6, 2-3 males, 2-3 females) and iE3/Cre^{+} mice (n=4-6, 2-3 males, 2-3 females). The amount of apoE in plasma and tissues in *APOE3*-TR mice (n=4, 2 males, 2 females) was also determined. The values in *APOE3*-TR mice were not included in the statistical analyses and are shown as references (D and E). *P<0.05, iE3/Cre^{-} vs. iE3/Cre^{+}, Mann-Whitney test. Data represent mean ± SEM.
(F and G) The amount of apoE in cortical tissues (F), and isolated brain vessels (G) was determined by ELISA in iE4/Cre^{-} (n=4-6, 2-3 males, 2-3 females) and iE4/Cre^{+} mice (n=4-6, 2-3 males, 2-3 females). The amount of apoE in plasma and tissues in *APOE4*-TR mice (n=4, 2 males, 2 females) was also determined. The values in *APOE4*-TR mice were not included in the statistical analyses and are shown as references (F and G). *P<0.05, iE4/Cre^{-} vs. iE4/Cre^{+}, Mann-Whitney test. Data represent mean ± SEM.
(H) Brain regional quantification of fluorescence intensity for GFP signals in brain sections from iE3/Cre^{+} mice (n=4, 2 males, 2 females). P=0.1143, cortex vs. hippocampus, Mann-Whitney test. Data represent mean ± SEM.
(I) Brain regional quantification of fluorescence intensity for GFP signals in brain sections from iE4/Cre^{+} mice (n=4, 2 males, 2 females). P=0.1143, cortex vs. hippocampus, Mann-Whitney test. Data represent mean ± SEM.

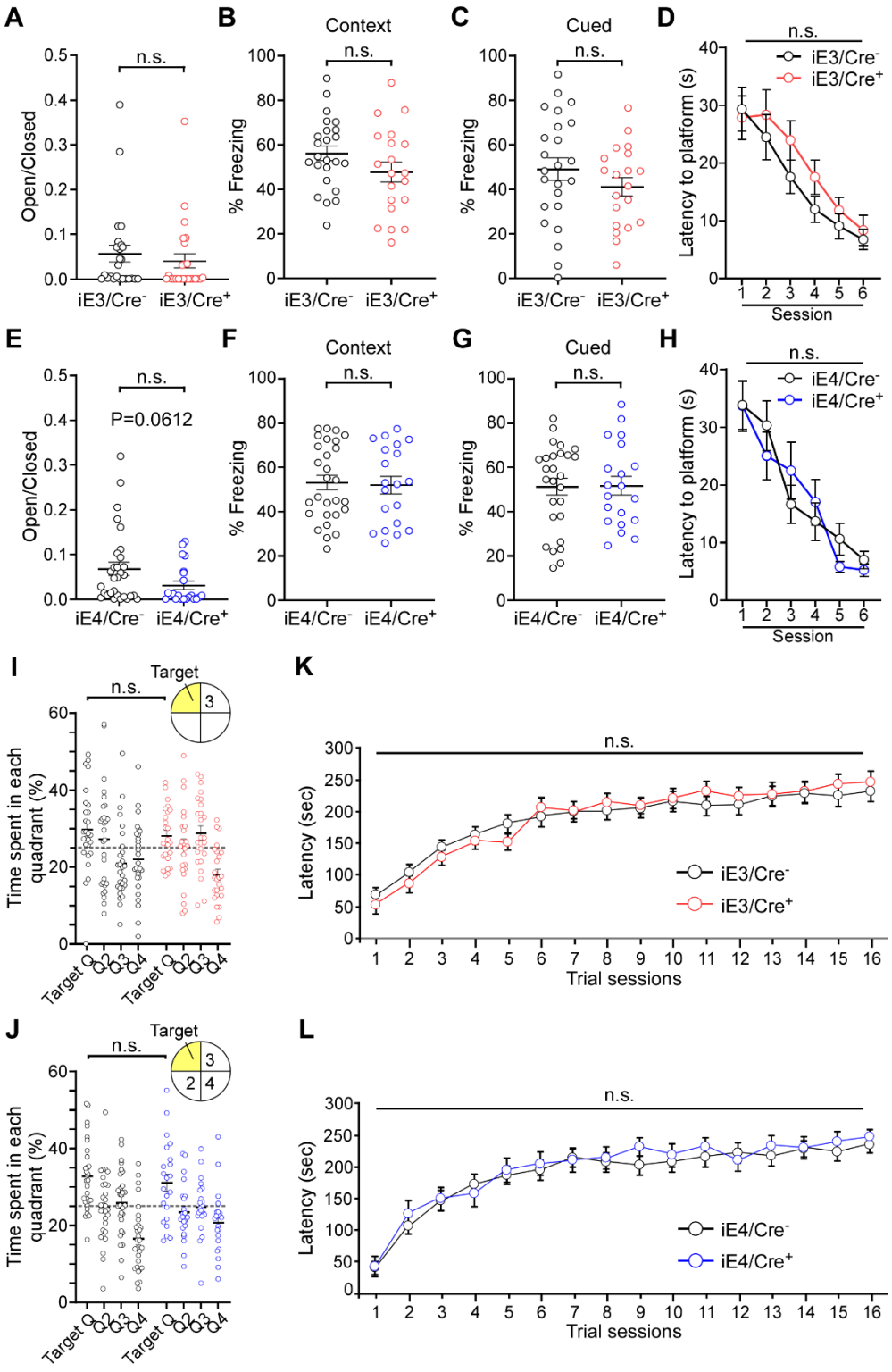

Figure S2. Behavior, memory performance, and motor coordination and balance in iE/Cre$^-$ and iE/Cre$^+$ mice, Related to Figure 2.

iE3/Cre$^-$ mice (n=28, 16 males, 12 females) and iE3/Cre$^+$ (n=26, 12 males, 14 females) littermates were behaviorally and cognitively assessed by elevated plus maze (EPM, A),

contextual and cued fear conditioning (CFC, B and C), and Morris Water Maze (MWM, D and I) tests at 6-8 months of age. Motor coordination and balance were assessed by rotarod test (J) at 6-8 months of age. Similarly, iE4/Cre$^-$ mice (n=30, 16 males, 14 females) and iE4/Cre$^+$ (n=22, 10 males, 12 females) littermates were behaviorally and cognitively assessed by EPM (E), CFC (F and G), and MWM (H and K) tests at 6-8 months of age. Motor coordination and balance were assessed by rotarod test (L) at 6-8 months of age.

(A) The ratio of the time spent in the open arms to that spent in the closed arms was used to examine the anxiety-like behavior. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.
(B and C) The hippocampus-dependent contextual phase of the CFC (B) and the hippocampus/amygdala-dependent cued phase of the CFC (C) were used to examine the associative learning and memory in iE3/Cre$^-$ and iE3/Cre$^+$ mice. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.
(D) iE3/Cre$^-$ and iE3/Cre$^+$ mice received 6 training sessions with the visible platform condition at Day 1 in MWM test. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$, repeated measures ANOVA. Data represent mean ± SEM.
(E) The ratio of the time spent in the open arms to that spent in the closed arms was used to examine the anxiety-like behavior. n.s., not significant, iE4/Cre$^-$ vs. iE4/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.
(F and G) The hippocampus-dependent contextual phase of the CFC (F) and the hippocampus/amygdala-dependent cued phase of the CFC (G) were used to examine the associative learning and memory in iE4/Cre$^-$ and iE4/Cre$^+$ mice. n.s., not significant, iE4/Cre$^-$ vs. iE4/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.
(H) iE4/Cre$^-$ and iE4/Cre$^+$ mice received 6 training sessions with the visible platform condition at Day 1 in MWM test. n.s., not significant, iE4/Cre$^-$ vs. iE4/Cre$^+$, repeated measures ANOVA. Data represent mean ± SEM.
(I, J) Time spent in “Target” quadrant during 60 seconds of MWM probe test (at Day 5) was used to examine the spatial learning in iE/Cre$^-$ and iE/Cre$^+$ mice. “Target” indicates the area where the platform was constantly located in the hidden-platform test. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$ (I) and iE4/Cre$^-$ vs. iE4/Cre$^+$ (J), Student t-test. Data represent mean ± SEM.
(K, L) Each mouse was placed on an accelerating spindle for 5 min for four consecutive trials. The latency to fall time was recorded in each trial from day 1 to day 4. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$ (K) and iE4/Cre$^-$ vs. iE4/Cre$^+$ (L), repeated measures ANOVA. Data represent mean ± SEM.

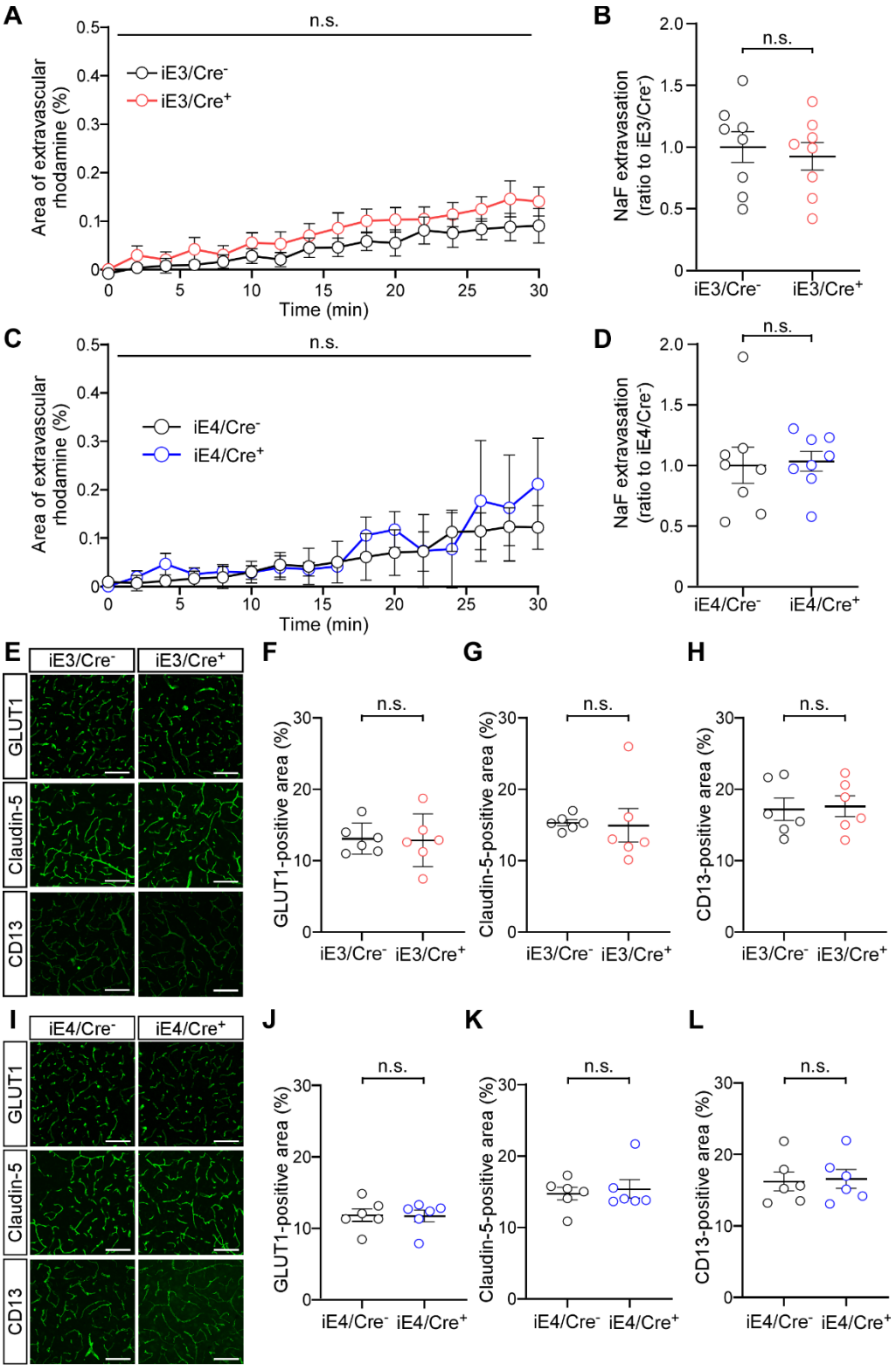

Figure S3. Functional and histological analyses of blood-brain barrier integrity in iE/Cre$^-$ and iE/Cre$^+$ mice, Related to Figure 3.

(A) Quantification of rhodamine-conjugated dextran extravasation in brain parenchyma. Rhodamine-conjugated dextran was intravenously injected in iE3/Cre$^-$ (n=3, male) and iE3/Cre$^+$ mice (n=3, male) and the extravasation of dextran as a measure of BBB leakage was monitored by two-photon microscopy. n.s., not significant, iE3/Cre$^+$ vs. iE3/Cre$^-$, repeated measures ANOVA. Data represent mean ± SEM.
(B) Quantification of sodium fluorescein (NaF) extravasation in brain parenchyma. A solution of 10% NaF was intraperitoneally injected in iE3/Cre$^-$ (n=8, 4 males, 4 females) and iE3/Cre$^+$ mice (n=8, 4 males, 4 females). The amount of NaF in cortices was calculated from a standard curve and normalized by the amount of tissues. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.
(C) Quantification of rhodamine-conjugated dextran extravasation in brain parenchyma. Rhodamine-conjugated dextran was intravenously injected in iE4/Cre$^-$ (n=3, male) and iE4/Cre$^+$ mice (n=3, male) and the extravasation of dextran as a measure of BBB leakage was monitored by two-photon microscopy. n.s., not significant, iE4/Cre$^-$ vs. iE4/Cre$^+$, repeated measures ANOVA. Data represent mean ± SEM.
(D) Quantification of NaF extravasation in brain parenchyma. A solution of 10% NaF was intraperitoneally injected in iE4/Cre$^-$ (n=8, 4 males, 4 females) and iE4/Cre$^+$ mice (n=8, 4 males, 4 females). The amount of NaF in cortices was calculated from a standard curve and normalized by the amount of tissues. n.s., not significant, iE4/Cre$^-$ vs. iE4/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.
(E) Brain sections from iE3/Cre$^-$ (n=6, 3 males, 3 females) and iE3/Cre$^+$ mice (n=6, 3 males, 3 females) were immunolabeled for endothelial cell (glucose transporter type 1, GLUT1, top), tight junction (claudin-5, middle) and pericyte (CD13, bottom) markers. Representative images are shown. Scale bar, 100 μm.
(F, G and H) Quantification of fluorescence intensity for GLUT1, claudin-5 and CD13 staining in brain sections from iE3/Cre$^-$ (n=6, 3 males, 3 females) and iE3/Cre$^+$ mice (n=6, 3 males, 3 females). n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.
(I) Brain sections from iE4/Cre$^-$ (n=6, 3 males, 3 females) and iE4/Cre$^+$ mice (n=6, 3 males, 3 females) were immunolabeled for endothelial cell (GLUT1, top), tight junction (claudin-5, middle) and pericyte (CD13, bottom) markers. Representative images are shown. Scale bar, 100 μm.
(J, K and L) Quantification of fluorescence intensity for GLUT1, claudin-5 and CD13 staining in brain sections from iE4/Cre$^-$ (n=6, 3 males, 3 females) and iE4/Cre$^+$ mice (n=6, 3 males, 3 females). n.s., not significant, iE4/Cre$^-$ vs. iE4/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.

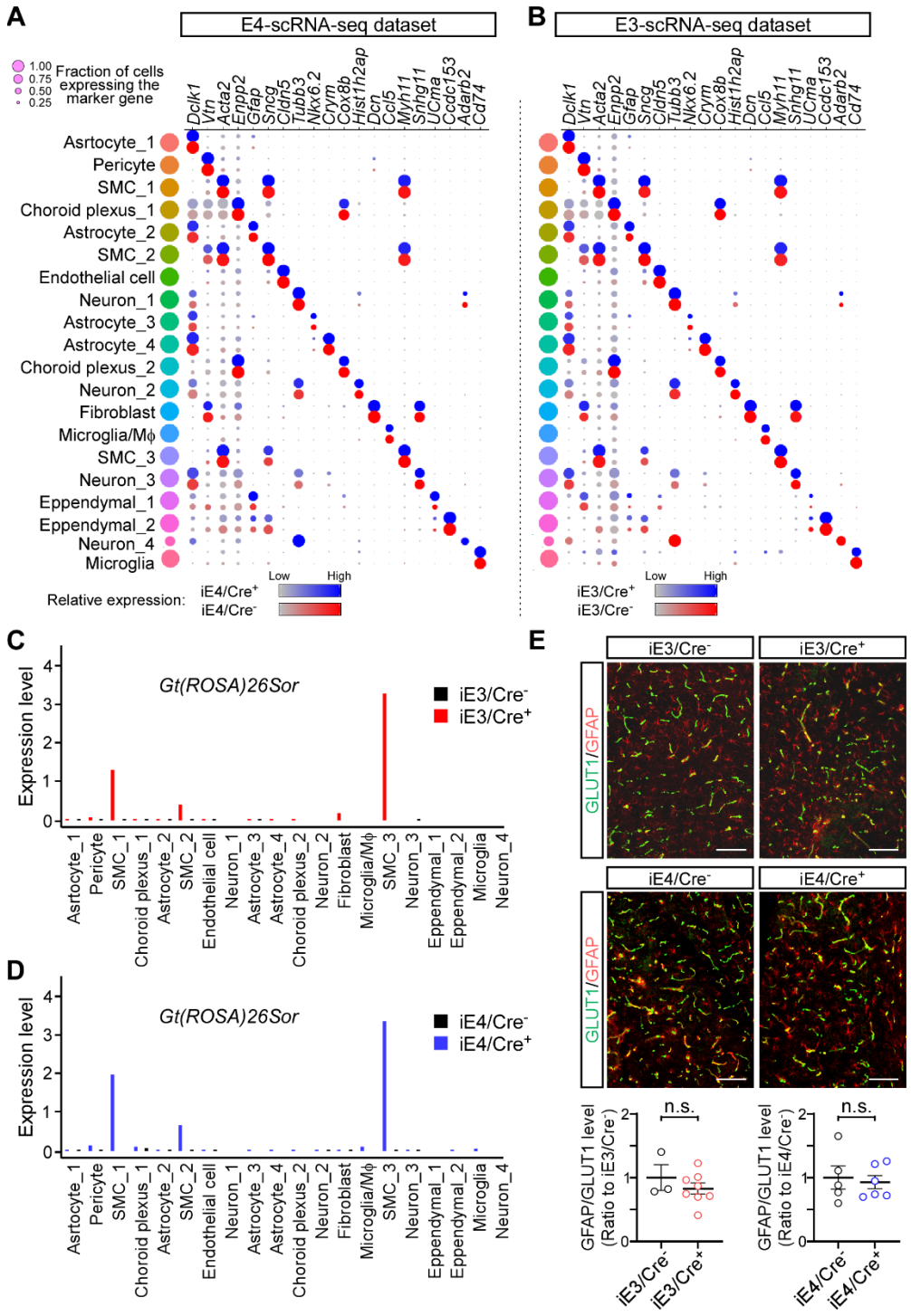

Figure S4. Additional analyses of glio-vascular phenotypes using scRNA-seq of vascular and glial cells isolated from iE/Cre$^-$ and iE/Cre$^+$ mice, Related to Figure 4.
(A and B) Split dot plot depicting marker genes for each cell population in E4-scRNA-seq (A) and E3-scRNA-seq (B) datasets. Marker genes were identified in an unbiased fashion blind to known cell type markers. The expression level (color intensity) and the percentage of cells in a cluster expressing any given gene (size) are reflected in circles as indicated at the upper-left

corner. Note that all cell populations except for “Neuron_4” are comprised from the comparable number of events from iE4/Cre^{+} (n=4, 2 males, 2 females) and iE4/Cre^{-} (n=4, 2 males, 2 females) mice in E4-scRNA-seq dataset. Similarly, cell populations except for “Neuron_4” are comprised from the comparable number of events from iE3/Cre^{+} (n=4, 2 males, 2 females) and iE3/Cre^{-} (n=4, 2 males, 2 females) mice in E3-scRNA-seq dataset. SMC, smooth muscle cell; MΦ, macrophage.

(C and D) Bar graph depicting the relative expression levels of *Gt(ROSA)26Sor* transcripts across cell types within glio-vascular unit. C, E3-scRNA-seq dataset, D, E4-scRNA-seq dataset.

(E) *Top*, coronal brain sections of iE3/Cre^{-}, iE3/Cre^{+}, iE4/Cre^{-}, and iE4/Cre^{+} mice containing the cortex and hippocampus were immunolabeled with GLUT1 and GFAP antibodies and the signals were visualized in green and red, respectively. Representative images are shown. Scale bar, 100 μm. *Bottom*, fluorescence intensity of GFAP signals surrounding the capillaries was quantified and normalized to the GLUT1 signals in coronal brain sections containing the cortex and hippocampus (n=3, 2 males, 1 female for iE3/Cre^{-}, n=8, 4 males, 4 females for iE3/Cre^{+}, n=5, 3 males, 2 females for iE4/Cre^{-}, n=6, 3 males, 3 females for iE4/Cre^{+}). n.s., not significant, iE3/Cre^{-} vs. iE3/Cre^{+} and iE4/Cre^{-} vs. iE4/Cre^{+}, Mann-Whitney test. Data represent mean ± SEM.

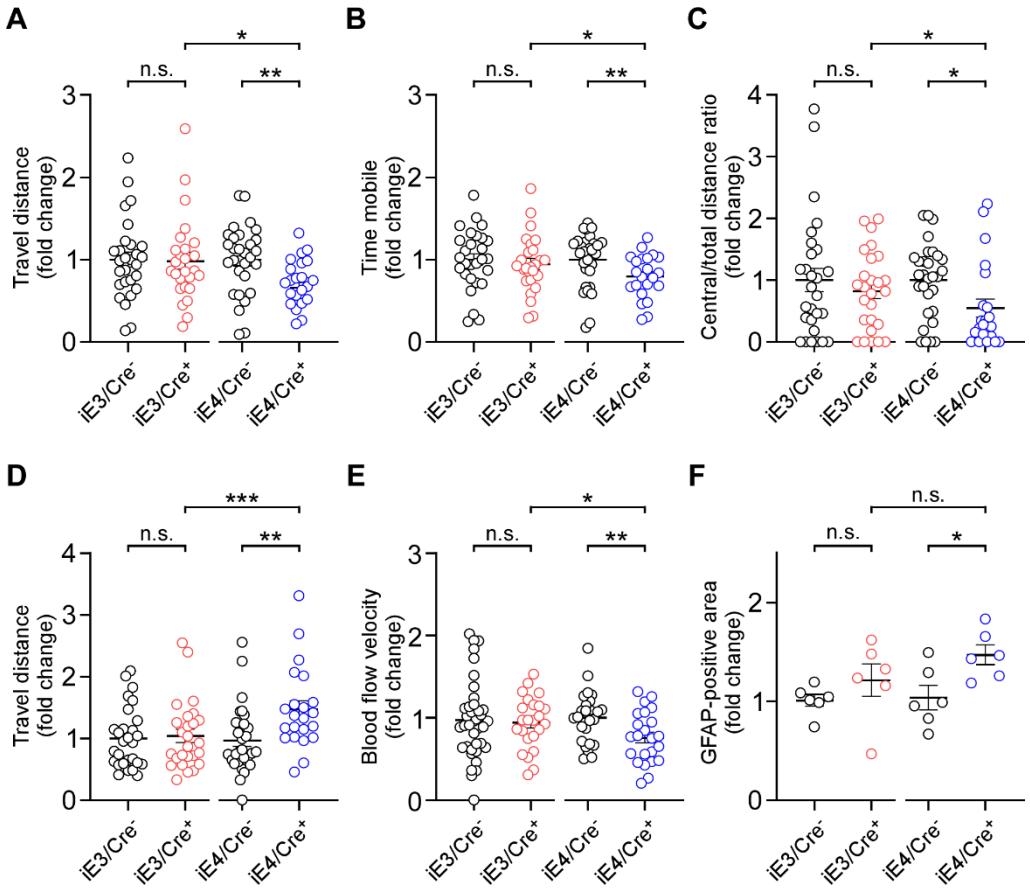

Figure S5. Side-by-side comparison of key readouts from iE/Cre$^-$ and iE/Cre$^+$ mice highlighting the differential effects of VMC-derived apoE3 or apoE4 on various phenotypes, Related to Figure 2-4.

(A, B and C) Related to **Figure 2A-F**. Total distance travelled (A) and time spent mobile (B) of iE/Cre$^-$ and iE/Cre$^+$ littermates in the OFA test were measured and plotted as magnitude of change. The center to total distance ratio (C) was used to examine the anxiety-like behavior. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$, Mann-Whitney test (A, C) and Student t-test (B). **P<0.01, iE4/Cre$^-$ vs. iE4/Cre$^+$, Student t-test (A) and Mann-Whitney test (B). *P<0.05, iE3/Cre$^+$ vs. iE4/Cre$^+$, Mann-Whitney test (A, C) and Student t-test (B). *P<0.05, iE4/Cre$^-$ vs. iE4/Cre$^+$, Mann-Whitney test (C). Data represent mean ± SEM.

(D) Related to **Figure 2G and 2I**. Travelled distances to platform of iE/Cre$^-$ and iE/Cre$^+$ littermates at the training Day 4 in the MWM test under the invisible platform condition were measured and plotted as magnitude of change. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$, Mann-Whitney test; **P<0.01, iE4/Cre$^-$ vs. iE4/Cre$^+$, Mann-Whitney test; ***P<0.001, iE3/Cre$^+$ vs. iE4/Cre$^+$, Mann-Whitney test. Data represent mean ± SEM.

(E) Related to **Figure 3A and 3B**. Mean arteriole RBC (red blood cell) flow was measured in iE/Cre and iE4/Cre$^+$ mice and plotted as magnitude of change. Each dot corresponds to one arteriole analyzed. n.s., not significant, iE3/Cre$^-$ vs. iE3/Cre$^+$, Mann-Whitney test; **P<0.01, iE4/Cre$^-$ vs. iE4/Cre$^+$, Student t-test; *P<0.05, iE3/Cre$^+$ vs. iE4/Cre$^+$, Student t-test. Data represent mean ± SEM.

(F) Related to **Figure 4G**. Fluorescence intensity of GFAP signals was quantified in coronal brain sections of iE3/Cre$^-$, iE3/Cre$^+$, iE4/Cre$^-$, and iE4/Cre$^+$ mice containing the posterior

cerebral artery and plotted as magnitude of change. n.s., not significant, iE3/Cre^{-} vs. iE3/Cre^{+}, Student t-test; *P<0.05, iE4/Cre^{-} vs. iE4/Cre^{+}, Student t-test; n.s., not significant, iE3/Cre^{+} vs. iE4/Cre^{+}, Student t-test. Data represent mean ± SEM.