Supplementary Material

Materials and Methods

Confocal imaging and analyses

For each mouse, 3 z-stack images were captured on the Nikon A1R$^+$ confocal microscope using 20x or 60x oil objective at 1024 × 1024 pixel resolution with a z-step size of 1.1 µm at 32 µm thickness. The same settings were used throughout an experiment. Acquired z-stack images were imported to Fiji software (ImageJ) and slices were merged into one stack (stack/Z projection) for each channel. For colocalization analysis, automatic thresholding method “Otsu” was set for plaques, microglia, and astrocytes, followed by colocalization between plaques and microglia or astrocytes using built-in image calculator AND operator. The percent of colocalization between the two channels was normalized to respective plaque load (total, parenchyma, or CAA). Images were processed and analyzed after blinding to treatment.

Cerebrovasculature isolation

As previously described with minor modifications *(50)*, immediately after transcardial perfusion, the forebrain was dissected and homogenized in a glass Dounce homogenizer (Kimble, 885300-0002) containing 20% w/v chilled vessel buffer (Hanks' Balanced Salt Solution, HBSS, HyClone, SH30268.02; 15 mM HEPES, Gibco, 15630080; 1 mM sodium pyruvate, Gibco, 11360070; 0.5% Bovine serum albumin, BSA, Fisher Scientific, 50550390; 1% dextran, Sigma-Aldrich, D8821). Next, vessels in vessel buffer were mixed with an equal volume of 31% dextran in water and centrifuged at 8000 x g for 30 min at 4 °C, which resulted in separation into three layers: vessel-free parenchyma (top), vessel-dextran solution with myelin/debris (middle), and vascular pellet (bottom). The parenchyma and myelin were removed and stored at –20 °C. To clean the vessels, three washes were performed using 0.5% BSA in HBSS. The well-resuspended vessel solution was further purified by passing over a 70 µm nylon mesh strainer

(Miltenyi Biotec, 130-098-462). Vessels were washed with 0.5% BSA in HBSS, centrifuged at 20,627 x g for 2 min at 4 °C in PBS, and stored at –80 °C. Vessels isolated from one forebrain of each mouse was considered an individual biological replica.

Immunohistochemistry and quantification

Brains were sectioned coronally at 50 µm using a freezing, sliding microtome (Leica) and stored in cryoprotectant solution (0.2 M PBS, 15% sucrose, 33% ethylene glycol) at –20 °C. For all histological studies unless otherwise stated, three sections were stained from each mouse separated by 300 µm (bregma −1.5, −1.8, and −2.1 mm) at room temperature and on a shaker as previously described*(19, 51)*. Antibodies for immunostaining for Aβ include biotinylated anti-$Aβ_{1-13}$ monoclonal antibody HJ3.4B (produced in-house, 2 µg/mL) and activated microglia using rat anti-CD45 (Biorad, MCA1388, 1:500) with goat anti-rat IgG secondary antibody (Life Technologies, A10517, 1:1000). Stained brain sections were scanned with a Nanozoomer 2.0-HT slide scanner (Hamamastu Photonics) and quantified using Fiji software version 1.52v (ImageJ). The percent area covered by staining was calculated by tracing the cortex overlaying the hippocampus. Three brain sections per mouse were averaged for an individual biological replica.

Live vascular imaging

Cerebrovascular function measurements were conducted as previously described*(23)* with a few modifications. Experiments were performed one week after the last i.p. injection. One femoral artery was cannulated for blood gases sampling (Nova Biomedical, BioProfile pHOx Analyzer) and a tracheostomy was performed under isoflurane (4% induction, 1.5% maintenance) for mechanical ventilation (Harvard Apparatus, MiniVent Ventilator) supplemented with 0.5% flow of 100% O_2. Fluorescein-dextran (Life Technologies, D1823, 150 uL at 12.5 mg/mL) was injected retro-orbitally for visualization of pial vessels. Mice were weaned off

isoflurane (a vasodilator) and anesthetized with pentobarbital which does not dilate vessels (i.p., 1.35 mL/kg from 50 mg/mL stock solution for first dose, 0.70 mL/kg for subsequent doses). For the craniotomy, mice were secured to a custom-built stereotaxic device (Instrument Machine Shop at Washington University School of Medicine) and the leptomeninges were exposed by gently removing the right parietal bone (4 mm in diameter) after drilling. A dental cement ring (Parkell, S380, 10 mm in diameter) surrounding the craniotomy was placed on the skull and filled with artificial cerebrospinal fluid (aCSF, in mM: 125 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 1 MgCl2, 1 CaCl2, and 25 mM glucose). To label fibrillar CAA, X34 dye was topically applied for 20 min to the cortical craniotomy. Next, the stage with the head-fixed mouse was secured to the Nikon Eclipse 600ME digital video microscopy system (Nikon Instruments Inc.). One vessel from a ramification of the middle cerebral artery (~20 μm in diameter) was imaged for the experiment if it contained both CAA-laden and CAA-free segments. Images were captured at 20x with water-immersion lenses at 1024 x 1024 pixels using MetaMorph imaging software version 7.10.2 (Molecular Devices). Two drugs were randomly assigned and topically applied to the window for 5 min: endothelium−dependent vasodilator acetylcholine (ACH; Sigma-Aldrich, A2661, 10 μM) or vascular smooth muscle cell−dependent vasodilator S−Nitroso−N−acetyl−penicillamine (SNAP; Sigma-Aldrich, N3398, 50 μM). To induce hypercapnia, mice were ventilated with 50% less volume and strokes per minute for 5 min. Images were captured at baseline and 5 min after each condition. After drug applications, aCSF was continuously infused for at least 30 min to allow vessel diameters to return to baseline. Baseline blood samples were collected at the start of the experiment and at the end of induced hypercapnia. Images were imported into Fiji (ImageJ) and the diameters of 6–8 ~30 μm vessel segments in length were measured with ObjectJ software plug-in version 1.04q. Data were calculated as percent vasodilatory change from baseline and grouped by amount of CAA coverage in the vessel segment. At least two vessel segments with less or more than 50% CAA

coverage were averaged per mouse. Vessel segments from one mouse were averaged and analyzed as an individual biological replica.

ELISA

Dissected posterior cortices were sequentially homogenized in chilled PBS containing protease inhibitors and 5 M guanidine-HCL buffer (pH 8.0) using magnetic beads (Next Advance, Bullet Blender Storm 24). Isolated vessel pellets were sonicated at 50% amplitude for 1 min total (1 s on, 1 s off) in 5 M guanidine-HCL solution. ELISAs were performed at room temperature unless otherwise stated. All antibodies were made in-house. The capture antibody for $A\beta_{40}$ was anti-$A\beta_{35\text{-}40}$ HJ2 and for $A\beta_{42}$ was anti-$A\beta_{37\text{-}42}$ HJ7.4. The detection antibody for $A\beta_{40}$ and $A\beta_{42}$ was biotinylated anti-$A\beta_{13\text{-}18}$ HJ5.1B. Aβ were performed as previously described and collected using Gen5 software version 1.11 with Synergy 2 (BioTek) *(19)*. Samples were loaded in duplicates, averaged per mouse for a biological replicate. ELISA data were normalized to total protein concentration using the Pierce Micro BCA Protein Assay Kit (Thermo Scientific, 23235) following manufacturer's instructions.

Gene expression assays

Total RNA was extracted from anterior mouse cortex using RNeasy Mini kit (Qiagen, 74106). Following the manufacturer's protocols, cDNA was prepared with the High-Capacity RNA-to-cDNA kit (Applied Biosystems, 4387406). Automated qPCR reactions using Taqman probes to determine relative gene expression were performed using Fluidigm Biomark HD by the Genome Technology Access Core at Washington University. Initial analyses were normalized to housekeeping gene *Gapdh* (chronic study) and subsequent analyses included the usage of more housekeeping genes (*Gapdh*, *Actin*, *Ywhaz*) to be normalized against using their geometric mean (acute study). Expression fold changes were normalized to control IgG and heatmaps were generated using Phantasus v1.5.1.

Supplementary Figures

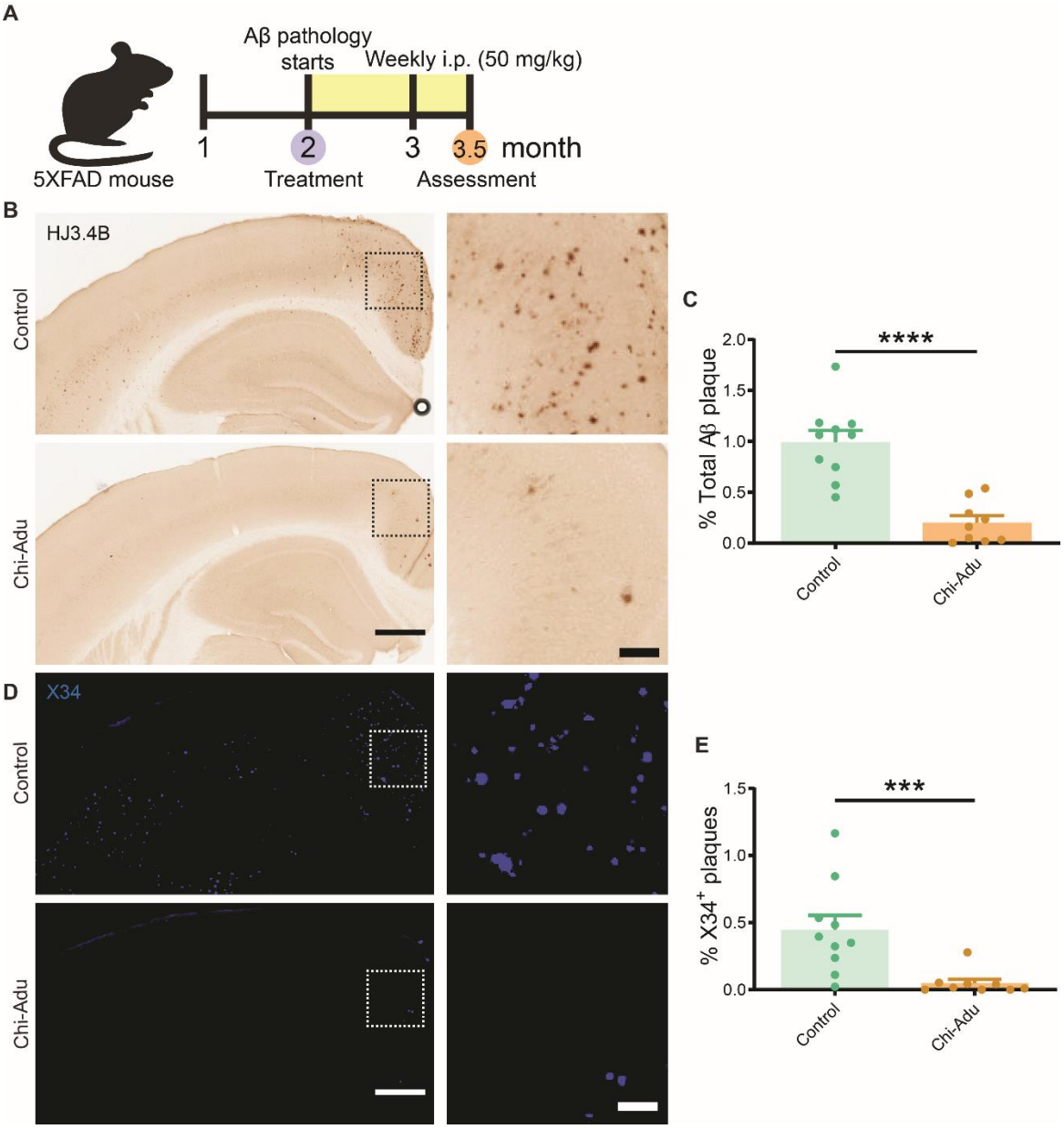

Fig. S1: Chi-Adu treatment reduces amyloid burden in a mouse model of parenchymal Aβ plaques. A, Schematic schedule of antibody treatment in 5XFAD (line 6799) mice expressing murine *Apoe* (Control IgG, n = 10; chi-Adu, n = 9). **B**, 3.5-month-old 5XFAD mouse brain sections stained with HJ3.4B for pan-Aβ and **C,** percent area quantification of parenchymal Aβ plaque coverage in cortex. **D**, **E**, Assessment of X34 coverage in cortex for fibrillar parenchymal plaques. Big panel: Scale bar = 500 µm. Small panel: Scale bar = 100 µm. Control = Control

IgG. Chi-Adu = Chimeric Aducanumab. Data expressed as mean ± SEM, student's *t*-test (two-sided). ***$P < 0.001$, ****$P < 0.0001$. No other statistical comparisons are significant unless indicated.

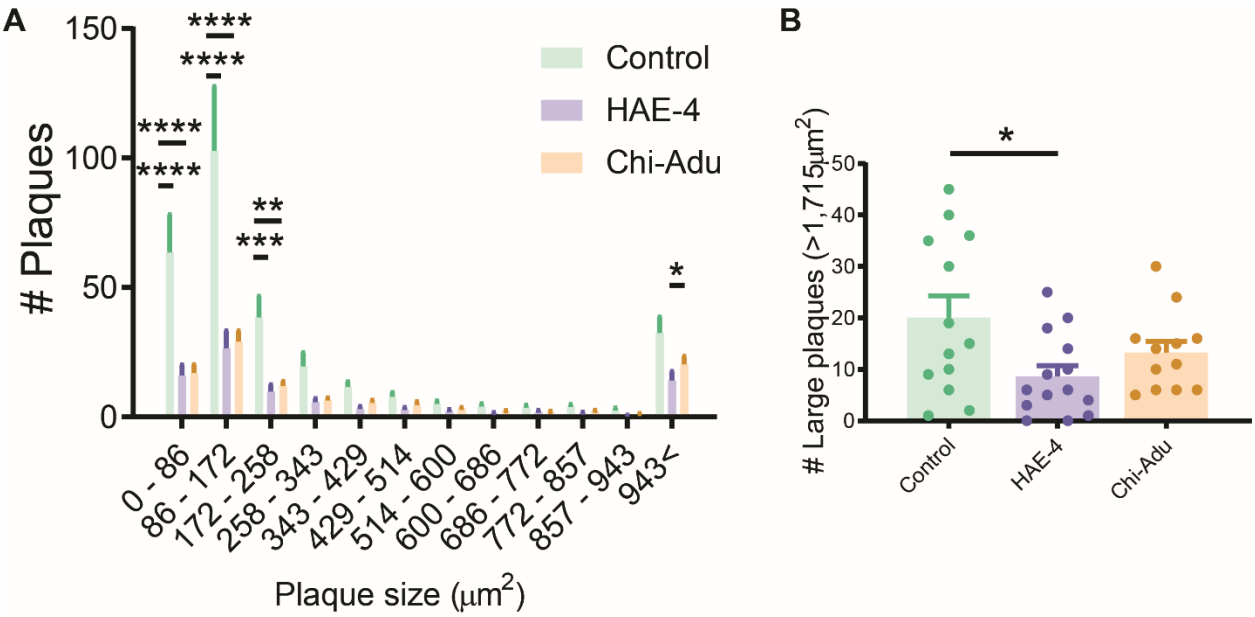

Fig. S2: HAE-4 strongly reduces Aβ parenchymal plaques of varying sizes in 5XE4 mice. **A**, Stratification of Aβ plaques by size for analysis of plaque distribution in cortex from 10-month-old antibody-treated 5XE4 mice (50 mg/kg, weekly i.p. for 8 weeks; Control IgG, n = 13; HAE-4, n = 14, chi-Adu, n = 12). Two-way ANOVA with Tukey's post hoc test (two-sided). **B**, Assessment of the number of large Aβ plaques (>1,715 μm^2). Control = Control IgG. Chi-Adu = Chimeric Aducanumab. Data expressed as mean ± SEM, one-way ANOVA with Tukey's post hoc test (two-sided). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. No other statistical comparisons are significant unless indicated.

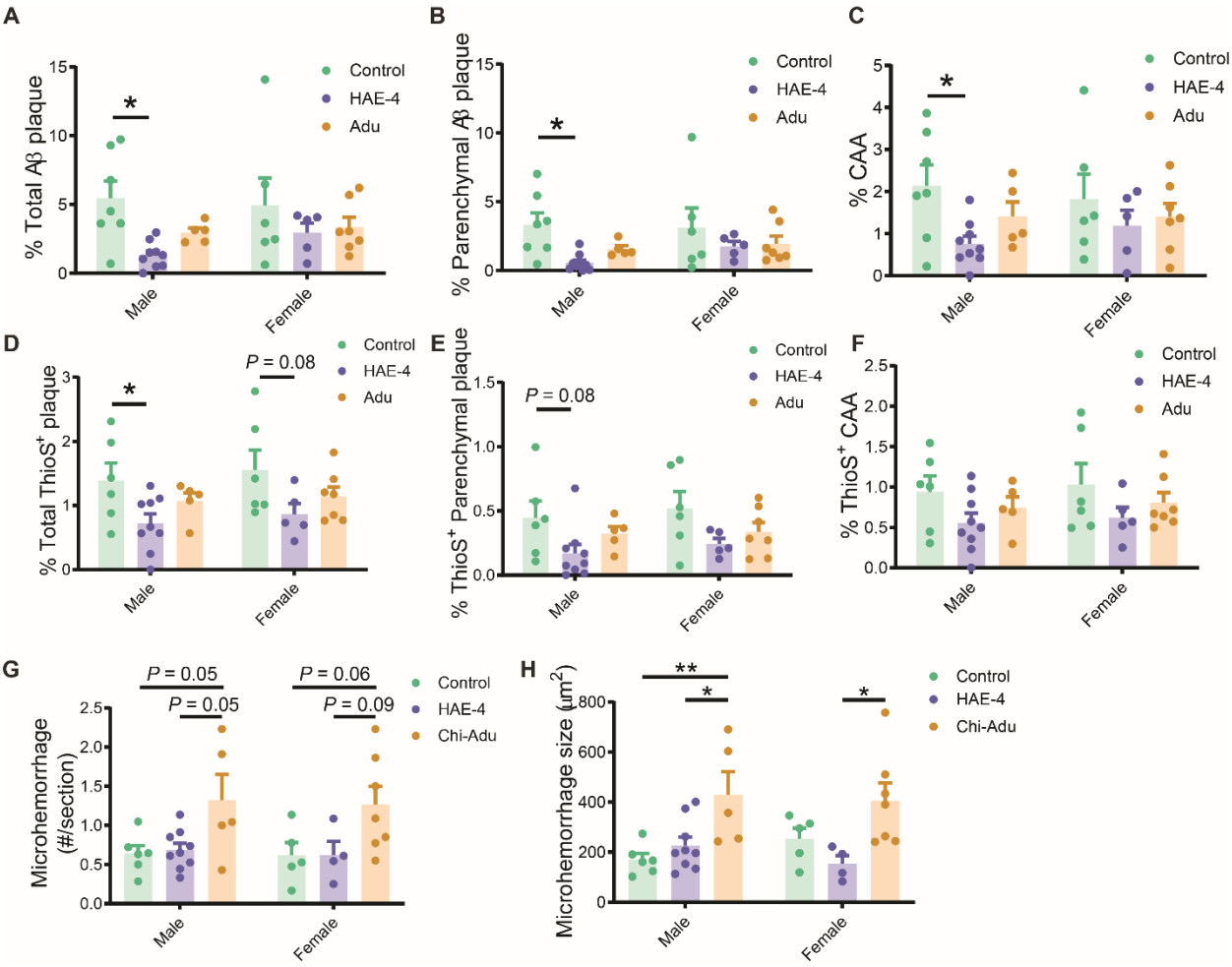

Fig. S3: No sex-dependent effect of HAE-4 or chi-Adu on Aβ plaque pathology or microhemorrhages. 10-month-old 5XE4 mice treated for 8 weeks were evaluated for sex-dependent differences in response to peripheral antibody administration (50 mg/kg, i.p., weekly; Control IgG, n = 11–12; HAE-4, n = 13, Adu, n = 12). **A–C**, Analysis of HJ3.4B immunoreactivity to determine percent coverage of total cortical Aβ (**A**: significant effect of treatment ($F_{2,33}$ = 4.585, *P* = 0.018) but no effect of sex ($F_{1,33}$ = 0.328, *P* = 0.571) or interaction ($F_{2,33}$ = 0.550, *P* = 0.582)), Aβ parenchymal (**B**: significant effect of treatment ($F_{2,33}$ = 4.242, *P* = 0.023) but no effect of sex ($F_{1,33}$ = 0.578, *P* = 0.453) or interaction ($F_{2,33}$ = 0.475, *P* = 0.626)), or CAA (**C**: significant effect of treatment ($F_{2,33}$ = 3.332, *P* = 0.048) but no effect of sex ($F_{1,33}$ = 0.009, *P* = 0.923) or interaction ($F_{2,33}$ = 0.452, *P* = 0.640)) pathology in male and female 5XE4 mice. **D–F**, Quantification of area covered by total ThioS (**D**: significant effect of treatment ($F_{2,32}$ = 5.432, *P*

= 0.009) but no effect of sex ($F_{1,32}$ = 0.553, P = 0.463) or interaction ($F_{2,32}$ = 0.026, P = 0.976)), parenchymal (**E**: significant effect of treatment ($F_{2,32}$ = 4.568, P = 0.018) but no effect of sex ($F_{1,32}$ = 0.524, P = 0.475) or interaction ($F_{2,32}$ = 0.066, P = 0.937)), and CAA (**F:** trend towards significant effect of treatment ($F_{2,32}$ = 2.854, P = 0.072) but no effect of sex ($F_{1,32}$ = 0.269, P = 0.608) or interaction ($F_{2,32}$ = 0.006, P = 0.995)) fibrillar plaques separated by sex. **G**, **H**, Prussian blue staining analysis segregated by sex for microhemorrhage frequency (**G**: significant effect of treatment ($F_{2,30}$ = 7.900, P = 0.002) but no effect of sex ($F_{1,30}$ = 0.095, P = 0.760) or interaction ($F_{2,30}$ = 0.007, P = 0.993)) and size (**H**: significant effect of treatment ($F_{2,30}$ = 10.27, P = 0.0004) but no effect of sex ($F1_{,30}$ = 0.008, P = 0.931) or interaction ($F_{2,30}$ = 1.005, P = 0.378)). Control = Control IgG. Chi-Adu = Chimeric Aducanumab. Data expressed as mean ± SEM, two-way ANOVA with Tukey's post hoc test (two-sided). *P < 0.05, **P < 0.01.

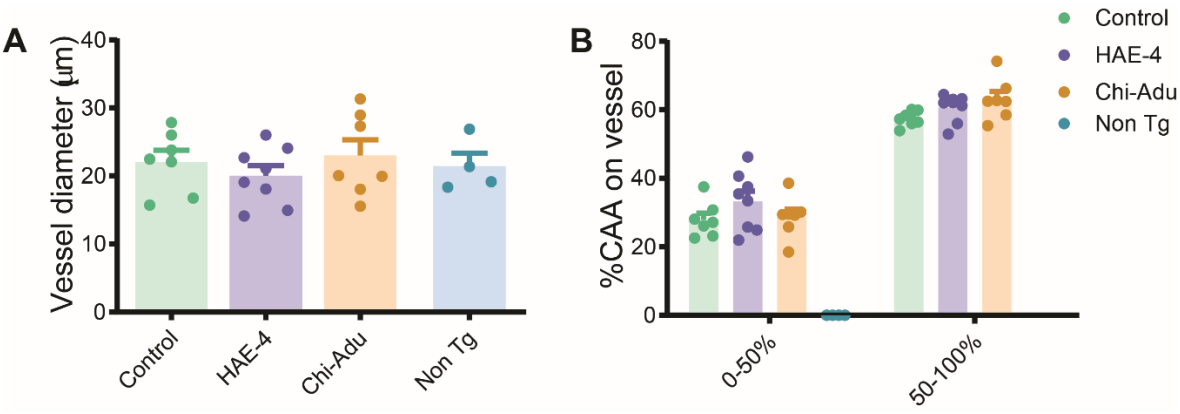

Fig. S4: Mean baseline vessel diameter and CAA coverage are consistent between treatment groups.

12-month-old 5XE4 mice were peripherally administered weekly antibody treatments prior to *in vivo* vascular imaging (50 mg/kg, i.p., 8 weeks, Control IgG, n = 7; HAE-4, n = 8, chi-Adu, n = 7, Non Tg, n = 4). **A**, **B**, Baseline leptomeningeal vessel diameter measurements (**A**; one-way ANOVA with Tukey's post hoc test, two-sided) and percent CAA on quantified vessel segments (**B**). Control = Control IgG. Chi-Adu = Chimeric Aducanumab. Data expressed as mean ± SEM, two-way ANOVA with Tukey's post hoc test (two-sided). No other statistical comparisons are significant unless indicated.

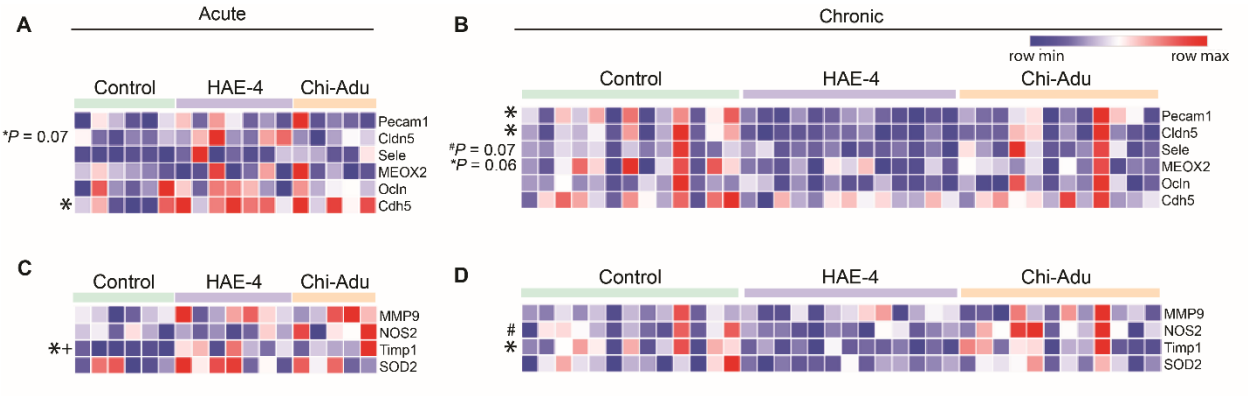

Fig. S5: Endothelial markers are initially upregulated but become quiescent with plaque removal after HAE-4 treatment.

A, **B**, Vascular transcripts of 11-month-old 5XE4 mice dosed acutely (**A**; 1X every 3 days for 4 total injections, 50 mg/kg, i.p.; Control IgG, n = 6; HAE-4, n = 7, chi-Adu, n = 5), or 10-month-old 5XE4 mice chronically receiving weekly systemic administrations (**B**; 8 weeks, 50 mg/kg, i.p.; Control IgG, n = 13; HAE-4, n = 13, chi-Adu, n = 12). **C, D**, Reactive oxygen species (ROS)-related gene expression changes in acute (**C**) or chronic (**D**) antibody treatments. "*" denotes statistical significance for HAE-4 versus control ($^{*}P < 0.05$); "#" for HAE-4 versus chi-Adu ($^{\#}P < 0.05$); "+" for chi-Adu versus control ($^{+}P < 0.05$). Control = Control IgG. Chi-Adu = Chimeric Aducanumab. Data expressed as mean ± SEM, one-way ANOVA with Tukey's post hoc test (two-sided). No other statistical comparisons are significant unless indicated.

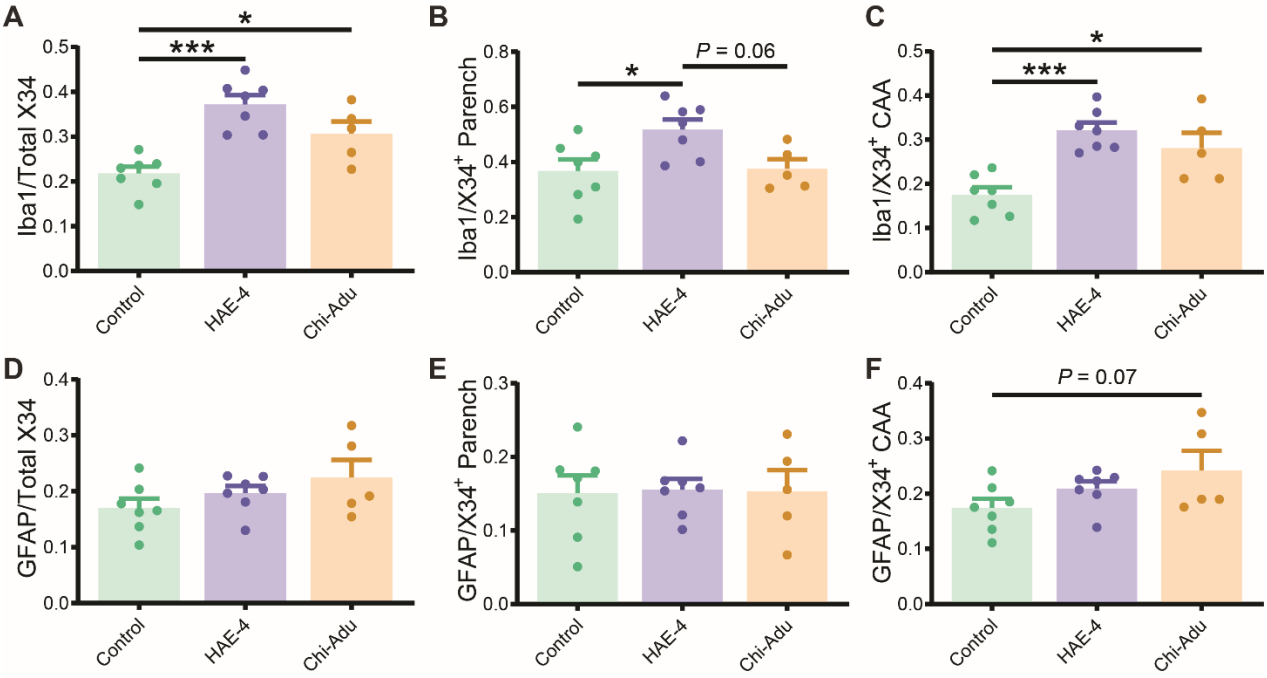

Fig. S6: Acute HAE-4 and chi-Adu treatments promote bidirectional glial clustering around Aβ plaques.

5XE4 at 11-month-of-age following acute antibody dosing study (1X every 3 days for 4 total injections, 50 mg/kg, i.p.) were assessed for percent glial colocalization with fibrillar Aβ plaques (Control IgG, n = 7; HAE-4, n = 7, chi-Adu, n = 5). **A–F**, Percent colocalization of Iba1+ microglia (**A–C**) or GFAP+ astrocytes (**D–F**) with total X34+ fibrillar plaques (**A, D**), in parenchyma (**B, E**) or CAA (**C, F**), normalized by plaque load. Parench = Parenchyma. Control = Control IgG. Chi-Adu = Chimeric Aducanumab. Data expressed as mean ± SEM, one-way ANOVA with Tukey's post hoc test (two-sided) and Dunnett's post hoc test for (**F**). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. No other statistical comparisons are significant unless indicated.

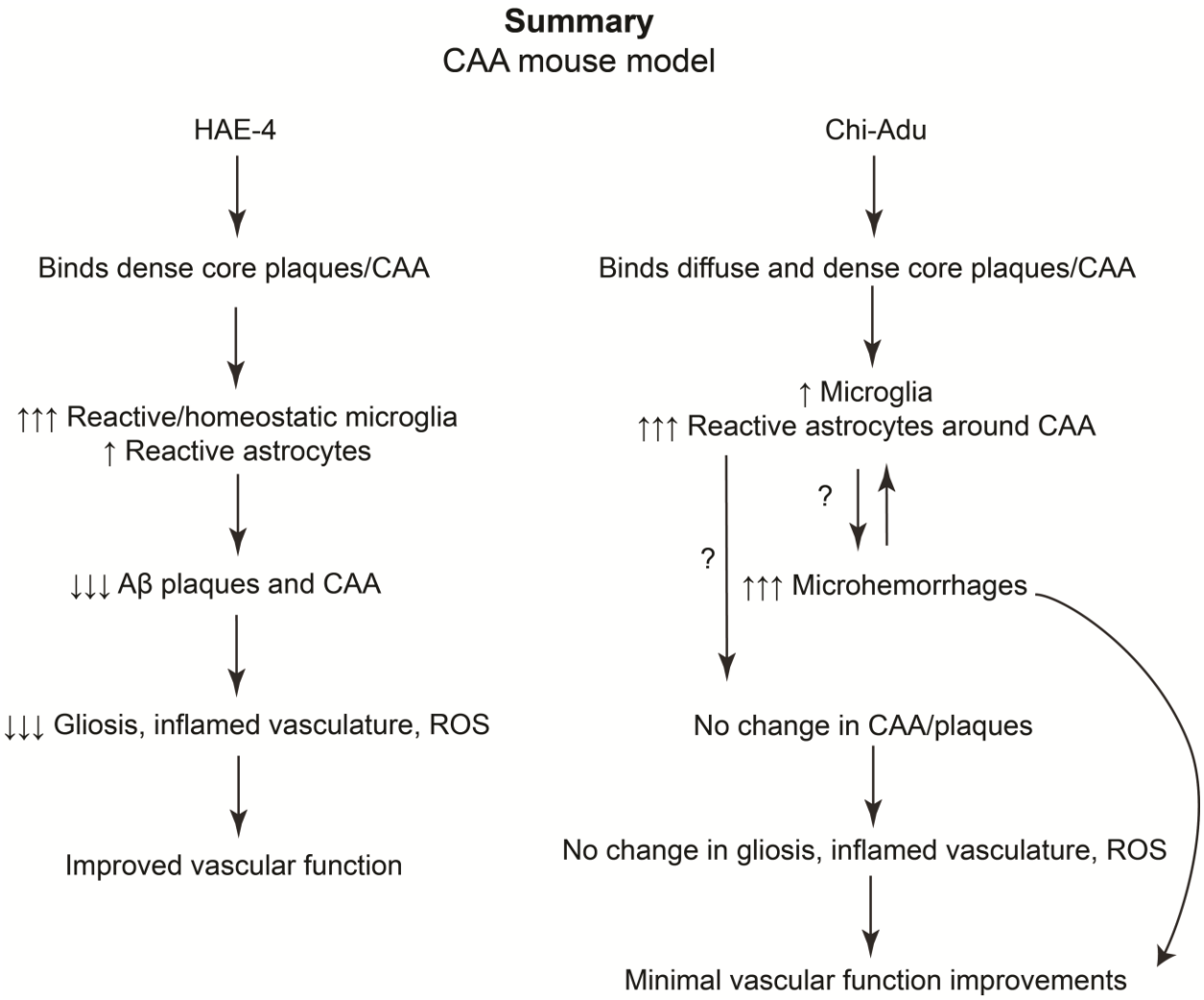

Fig. S7: Schematic summary of differential HAE-4 and chi-Adu antibody effects in a mouse model with robust CAA. HAE-4 preferentially binds to APOE within dense core plaques/CAA. Initially, HAE-4 stimulates mostly microglia and, to a lesser extent, astrocytes to cluster around plaques/CAA. This leads to reduced amyloid burden and ameliorates downstream plaque-induced gliosis/inflammation, providing a favorable microenvironment for restoring cerebrovascular function. In a context with prominent CAA, chimeric Aducanumab (chi-Adu), which binds to both diffuse and dense core plaques/CAA, initially elicits some microgliosis and more astrocytosis. Reactive astrocytes particularly around blood vessels with CAA may lose their function in maintaining the blood-brain barrier, leading to increased hemorrhages. Alternatively, chi-Adu directly promotes microhemorrhages by an unknown

mechanism to allow for the influx of toxic blood proteins or infiltrating immune cells that may promote severe astrogliosis around CAA. The recruitment of less microglia by chi-Adu may also hinder plaque clearance, leading to limited reduction in gliosis and inflammation and consequently minimal improvements in vascular function.

Table S1.

Pathology	Sex	*APOE* genotype	Braak	PMI	Age
Control	F	E2/E2	III	2.5	91
CAA	M	E4/E4	IV	2	87
AD	F	E3/E3	IV	3.16	93

Table. S1: Demographics of human brain tissue. Relevant variables of patient brain tissue with CAA, AD, or no pathology stained with X34, HJ3.4, HAE-4, or chimeric Aducanumab (**Fig. 2**).

Table S2.

Baseline

	Control	HAE-4	Chi-Adu	Non Tg
pH	7.45 ± 0.02	7.45 ± 0.02	7.47 ± 0.03	7.44 ± 0.02
pCO_2 (mmHg)	37.4 ± 0.98	35.0 ± 1.79	32.2 ± 1.21	31.1 ± 2.28
SO_2%	99.5 ± 0.32	99.6 ± 0.13	99.6 ± 0.14	99.7 ± 0.20

Hypercapnia

	Control	HAE-4	Chi-Adu	Non Tg
pH	7.19 ± 0.03	7.24 ± 0.01	7.28 ± 0.02	7.26 ± 0.02
pCO_2 (mmHg)	60.22 ± 1.12	59.8 ± 1.54	58.2 ± 3.06	61.7 ± 0.96
SO_2%	99.2 ± 0.52	97.9 ± 1.20	99.3 ± 0.25	99.3 ± 0.38

Table. S2: No difference in blood gas measurements at baseline and hypercapnia. 12-month-old 5XE4 mice treated weekly with antibodies for live imaging (50 mg/kg, i.p., 8 weeks, Control IgG, n = 7; HAE-4, n = 8, chi-Adu, n = 7, Non Tg, n = 4). Blood gases were regulated and samples with measurements for pH, pCO_2, and SO_2 were collected before baseline imaging and during hypercapnia. Control = Control IgG. Chi-Adu = Chimeric Aducanumab. Data expressed as mean ± SEM, one-way ANOVA with Tukey's post hoc test (two-sided). No statistical comparisons are significant.

Data file S1. Primary data. Excel file provided.