Supporting Information

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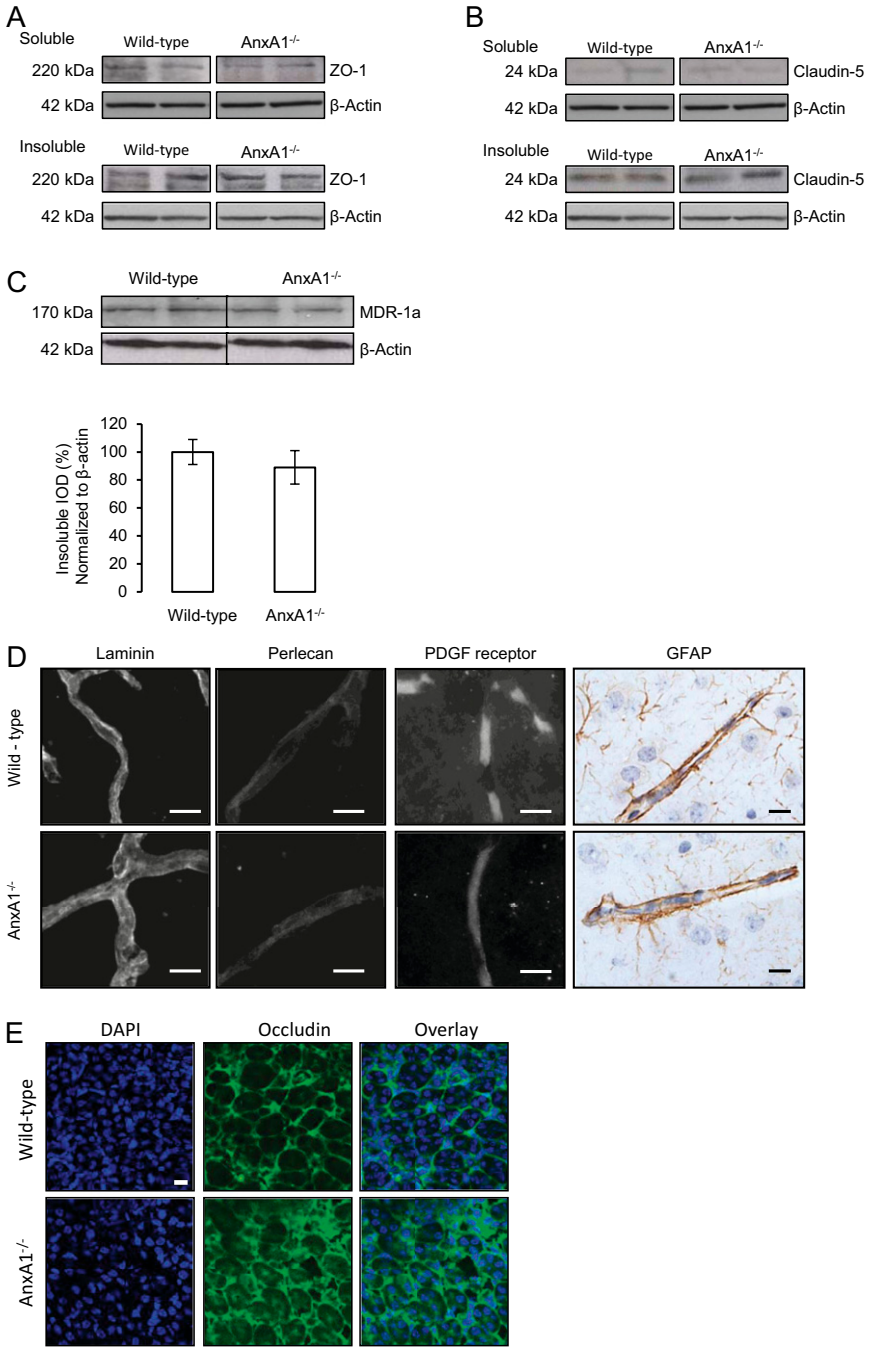

Fig. S1. Analysis of blood–brain barrier components in WT and annexin A1 (AnxA1)-null mice. (*A* and *B*) Typical Western blot analysis of soluble and insoluble zonula occludens-1 (ZO-1; *A*) and claudin-5 (*B*) content in cortical extracts from WT and AnxA1$^{-/-}$ mice, alongside β-actin loading controls (n = 3 independent experiments, each with n = 4 male samples per genotype). (*C*) Typical Western blot analysis of MDR-1a (p-glycoprotein) expression in cortical extracts from WT and AnxA1$^{-/-}$ mice, alongside actin loading controls. The histogram shows densitometric analysis of four animals per genotype, expressed as mean ± SEM. (*D*) Confocal microscopic analysis of the typical blood–brain barrier basement membrane proteins laminin and perlecan, the pericyte marker, the PDGF receptor, and bright-field microscopic analysis of the astrocyte marker GFAP in WT and AnxA1$^{-/-}$ mice (typical examples from n = 6 mice per genotype). (Scale bars: 10 μm.) (*E*) Confocal microscopic analysis of occludin expression in the kidney from WT and AnxA1$^{-/-}$ mice (typical examples from n = 3 mice per genotype). (Scale bar: 20 μm.)

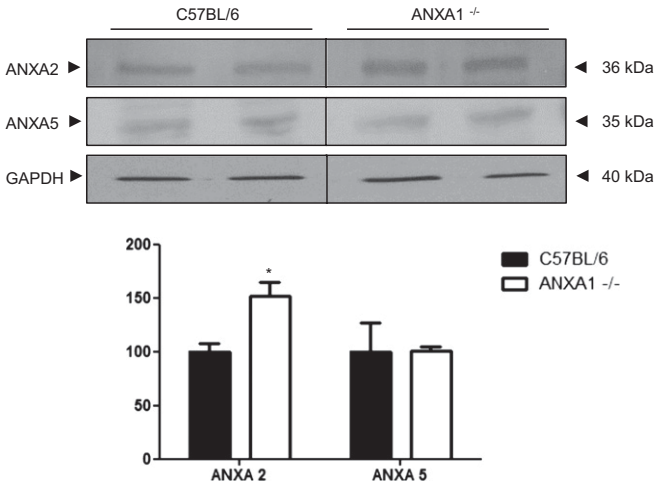

Fig. S2. Analysis of compensatory changes in annexin A2 and annexin A5 in the cerebral microvasculature of AnxA1$^{-/-}$ mice. Typical Western blot analysis of annexin A2 (AnxA2; *Upper*) and annexin A5 (AnxA5; *Lower*) expression in extracts from isolated cerebral capillaries of WT and AnxA1$^{-/-}$ mice, alongside GAPDH loading controls ($n = 3$ independent experiments, each with $n = 4$ male samples per genotype). Histograms show densitometric analysis of four animals per genotype, expressed as mean ± SEM.

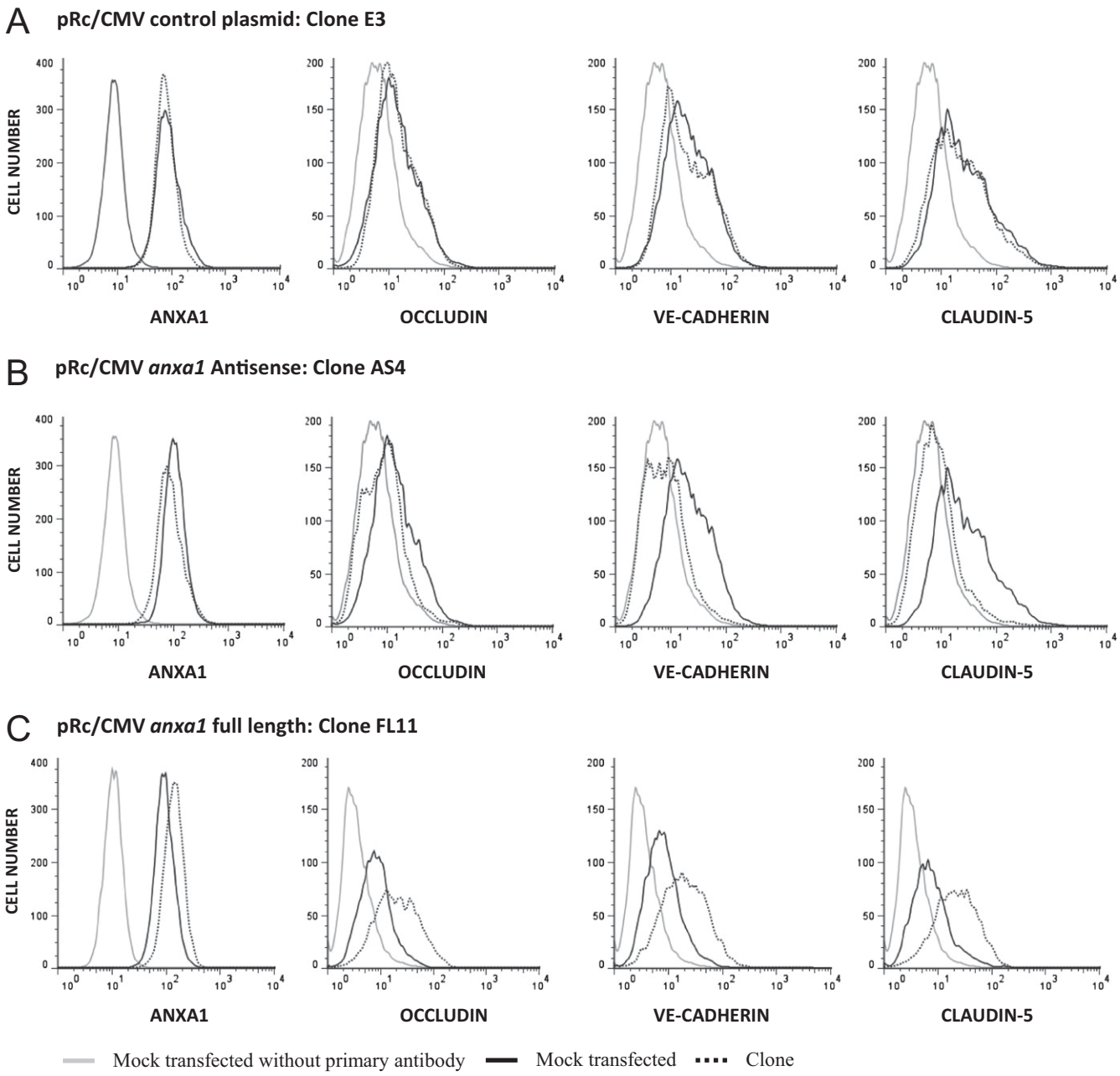

Fig. S3. Immunophenotyping of stable hCMEC/D3 clones. (*A*–*C*) Representative examples of flow cytometric analyses of ANXA1, occludin, vascular endothelial (VE)-cadherin, and claudin-5 expression in hCMEC/D3 clones stably transfected with a pRc/CMV control plasmid (*A*), a pRc/CMV plasmid bearing an antisense sequence for *anxa1* (*B*), or a pRc/CMV plasmid bearing the full-length sequence for *anxa1* (*C*). At least 10,000 events were analyzed per profile, and histograms represent one of at least three independent experiments.

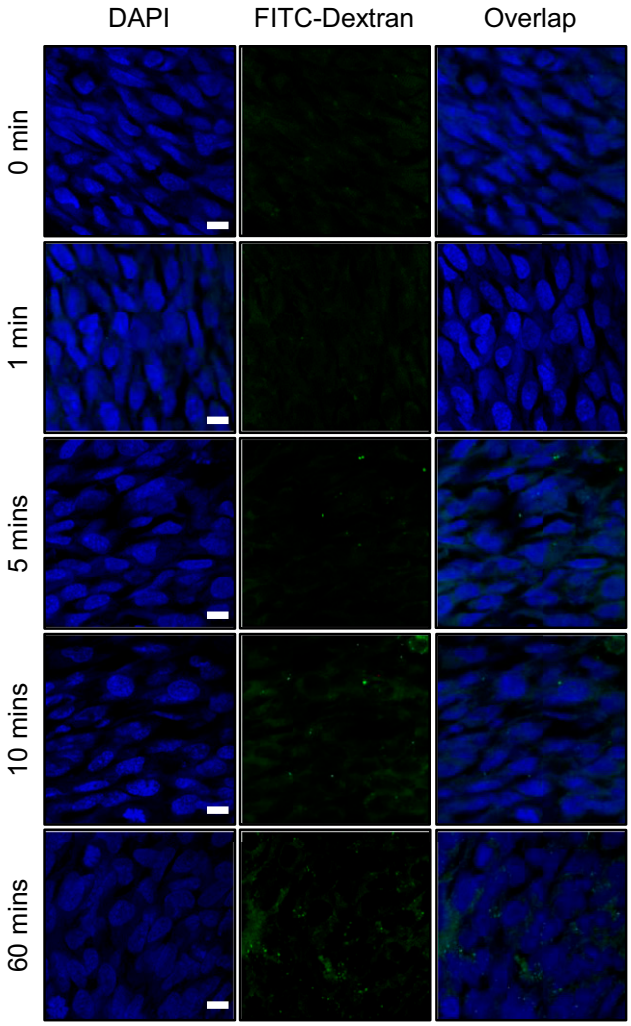

Fig. S4. Assessment of endocytosis of 70-kDa FITC-dextran by hCMEC/D3 monolayers: Confocal microscopic analysis of confluent monolayers of WT hCMEC/D3 cells incubated for various time points with a 3-mg/mL solution of 70 kDa FITC-dextran. *Z*-stack profiles were recorded and analyzed for presence of endocytotic cytoplasmic vesicles. No evident sign of significant endocytosis was detected at any time point, indicating that the movement of the fluorescent molecules from apical to basolateral regions of the monolayer occurs mainly through the paracellular pathway. Pictures are representative of three samples per time point and of all hCMEC/D3 clones used. (Scale bar: 5 μm.)

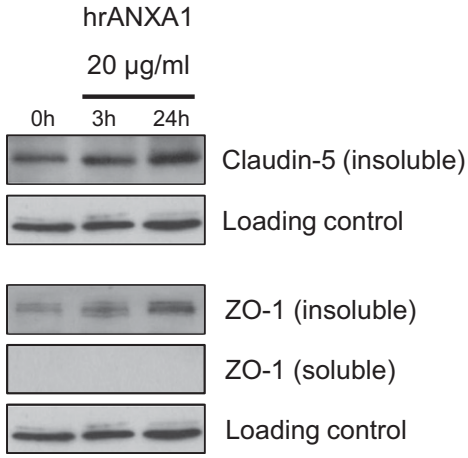

Fig. S5. Exogenous ANXA1 effects on tight junctions. Administration of 20 μg/mL human recombinant ANXA1 to WT hCMEC/D3 cells induces an up-regulation of membrane-associated (i.e., insoluble) zonula occludens-1 (ZO-1) and claudin-5 expression as measured by Western blot. Data are representative of at least three independent experiments.

Table S1. Human tissue demographic data

Disease	No. of cases	Mean age (range), y	Mean disease duration (range), y	Sex ratio, M:F	Postmortem delay, h
Multiple sclerosis	11	63.2 (43–81)	30 (14–50)	4:7	9–27
Parkinson disease	7	73.7 (62–87)	13 (5–27)	5:2	7–26
Nonneurologic control	6	83.6 (81–88)	NA	3:3	7–47

Information concerning postmortem brain tissue used to assess changes in ANXA1 expression at the level of the microvasculature. NA, not applicable.

Table S2. Human plasma demographic data

Patient no.	Age, y	Sex	MS type	Treatment
BUH00114	71	M	SP	Rebif
BUH00115	49	M	R/R	None
BUH00116	45	F	R/R	None
BUH00117	49	M	R/R	Copaxone
BUH00118	64	F	PP	Avonex
BUH00119	65	F	R/R	None
BUH00120	56	F	R/R	Rebif
BUH00121	44	F	R/R	Rebif
BUH00122	51	F	R/R	Rebif
BUH00123	60	F	SP	Rebif

MS, multiple sclerosis; PP, primary progressive; R/R, relapsing/remitting; SP, secondary progressive. Rebif and Avonex are IL-1βa. Copaxone is glatiramer acetate. All patients are of white race. Control samples are from 10 disease-free age- and sex-matched donors.