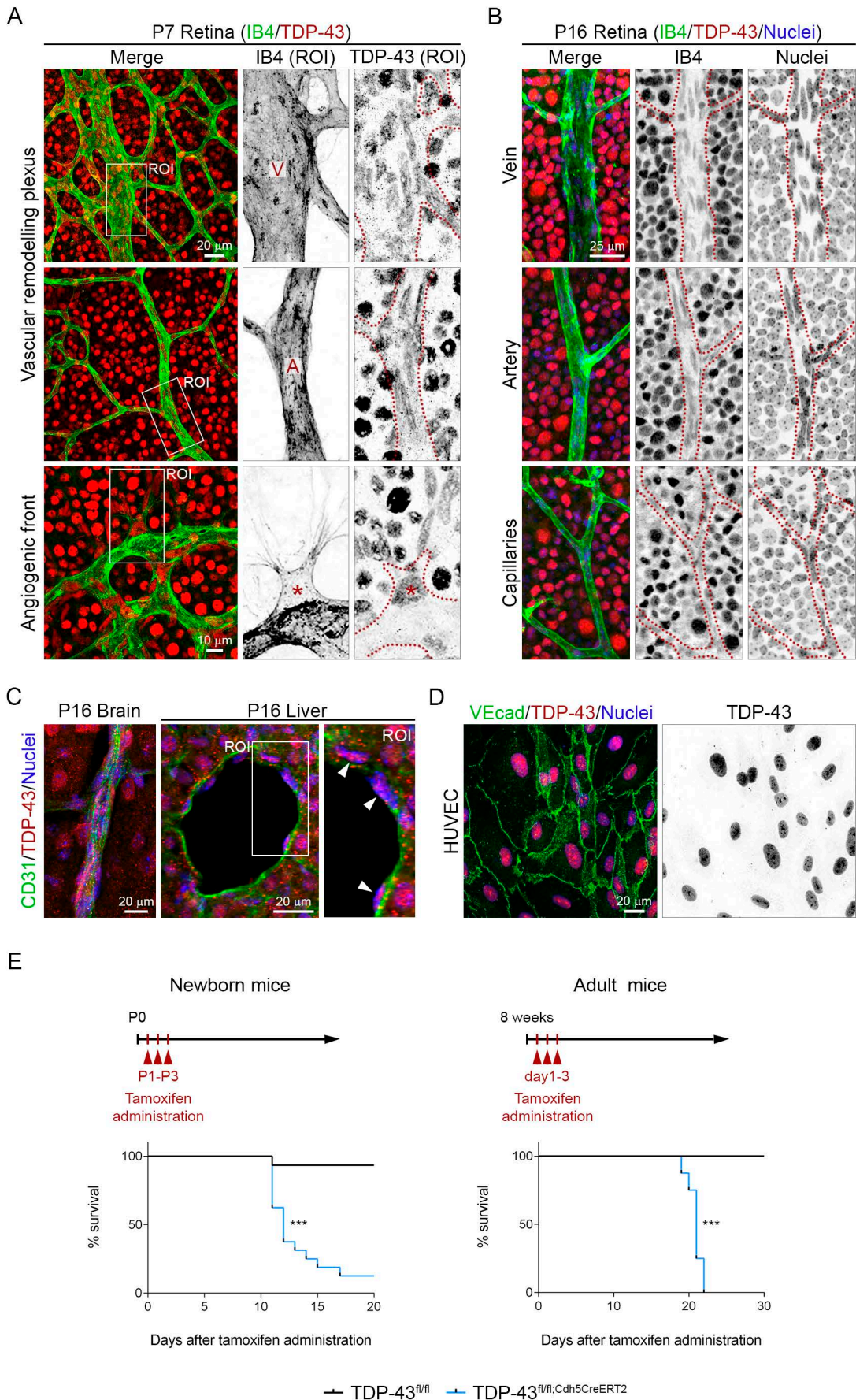


Supplemental Materials

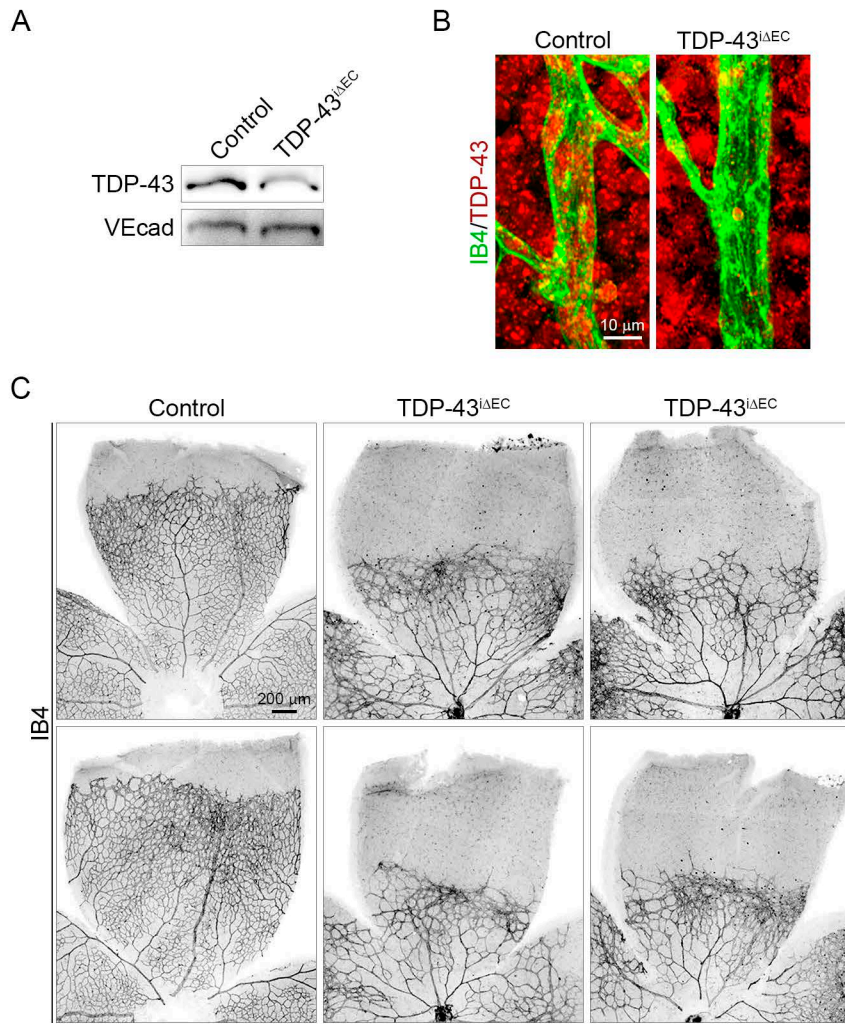
Endothelial TDP-43 controls sprouting angiogenesis and vascular barrier integrity, and its deletion triggers neuroinflammation

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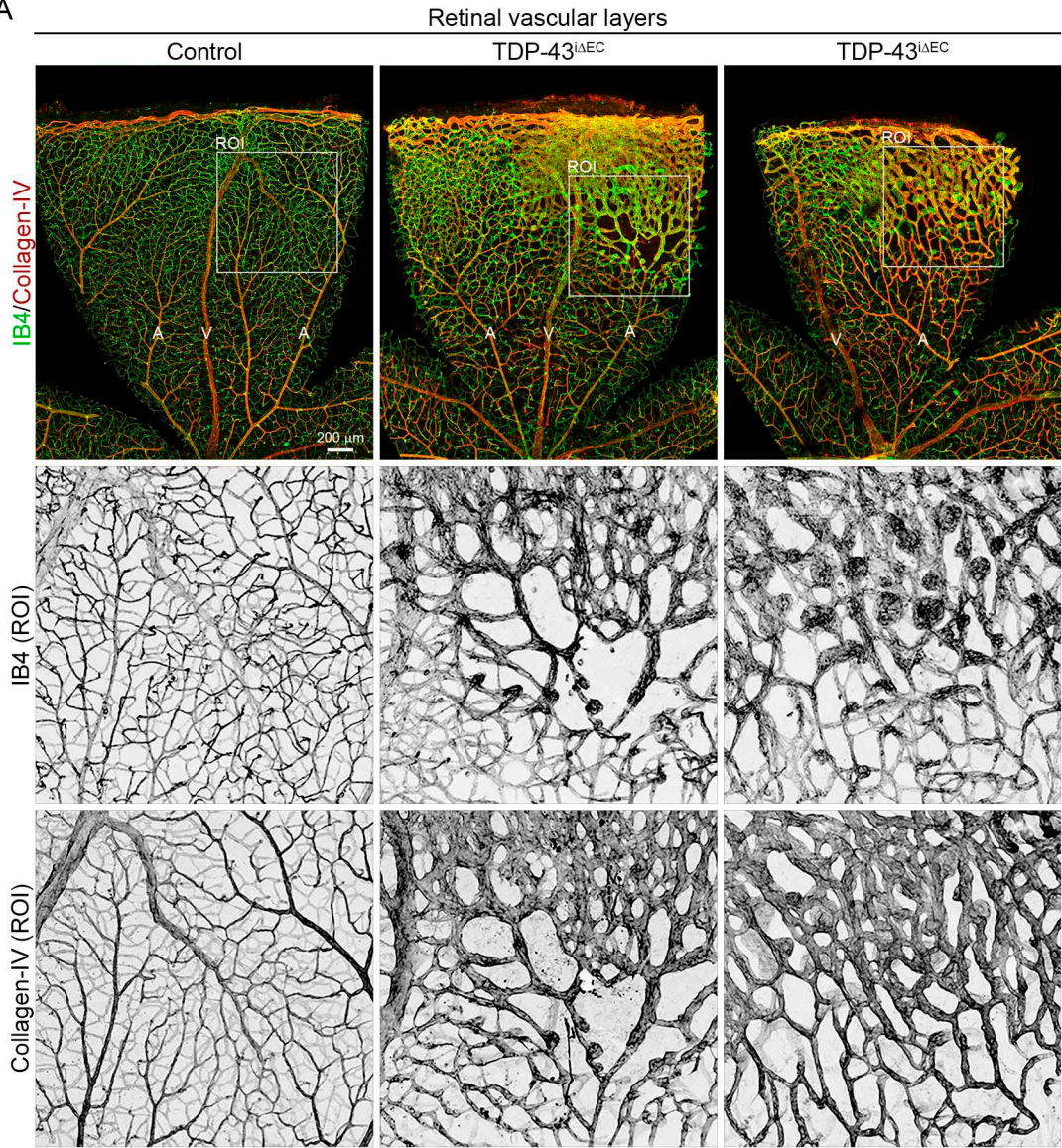


Supplemental Figure 1. Expression of TDP-43 in the vasculature. Confocal high-magnification images of P7 (A) and P16 (B) wild-type mouse retinas stained for IB4 (green), TDP-43 (red) and Hoechst (nuclei, blue). White rectangles indicate the magnified ROIs. Dotted lines highlight the endothelial border of the blood vessels. Veins (V), arteries (A) and sprouting ECs (asterisks) are indicated. (C) Confocal high-magnification images of P16 control mouse brain and liver sections stained for CD31 (green), TDP-43 (red) and Hoechst (nuclei, blue). White rectangle indicate the magnified ROI. (D) Confocal high-magnification images of HUVECs stained for VEcad (green), TDP-43 (red) and Hoechst (nuclei, blue). (E) Kaplan-Meier survival curve for newborn and adult *TDP-43^{fl/fl}* and *TDP-43^{fl/fl};Cdh5CreERT2* mice. p values (***) $p < 0.001$. Log-rank (Mantel-Cox) test.

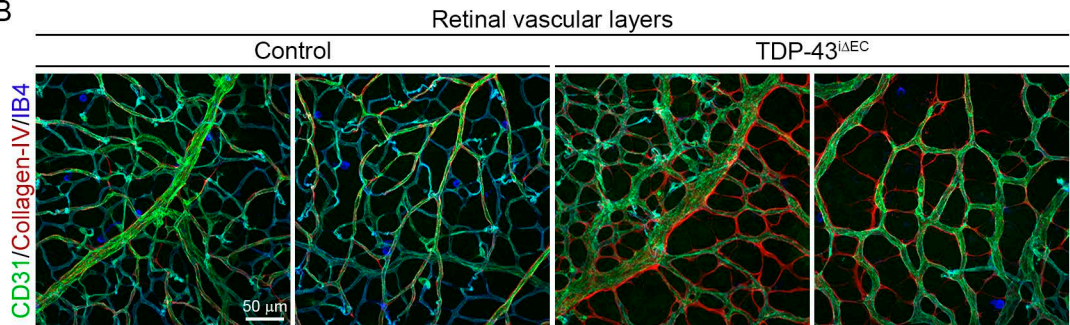


Supplemental Figure 2. *Cdh5(PAC)-Cre^{ERT2}*-mediated deletion of *Tdp-43* gene. (A) Western blot analysis of TDP-43 in lung lysates from P6 control and *TDP-43^{iΔEC}* mice three days after tamoxifen administration. VEcad was used as a loading control. (B) Confocal high-magnification images of P7 control and *TDP-43^{iΔEC}* retinas stained for IB4 (green) and TDP-43 (red). (C) Confocal high-magnification images of P7 control and *TDP-43^{iΔEC}* retinas stained for IB4.

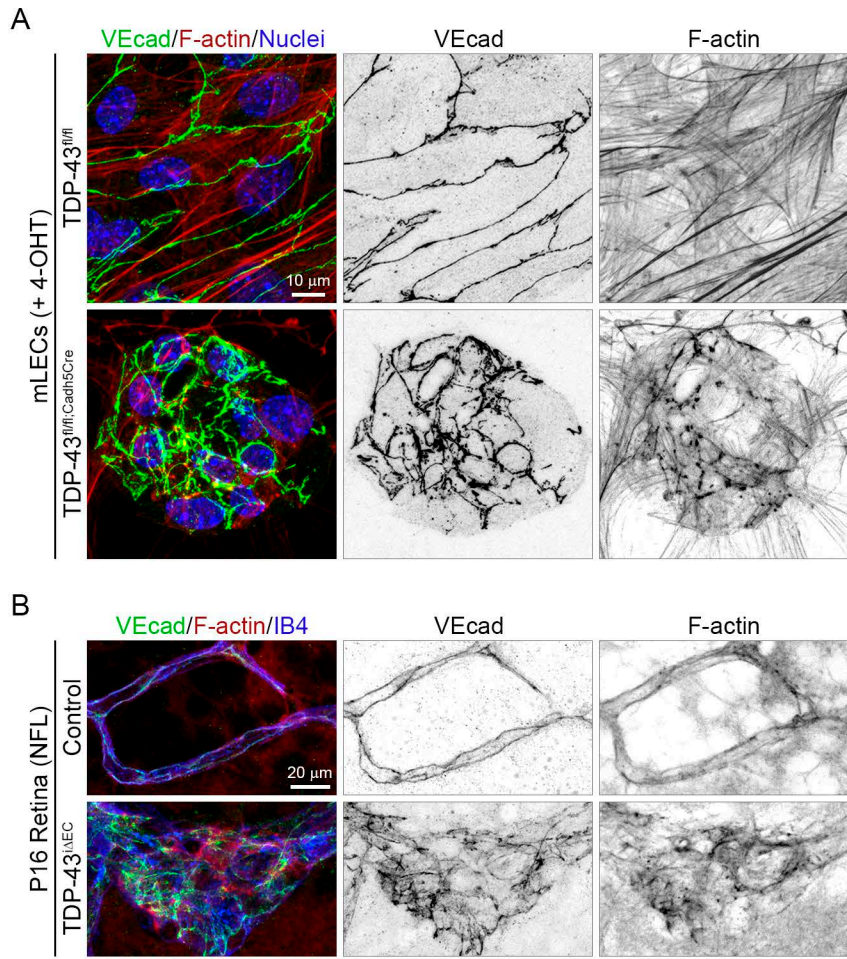
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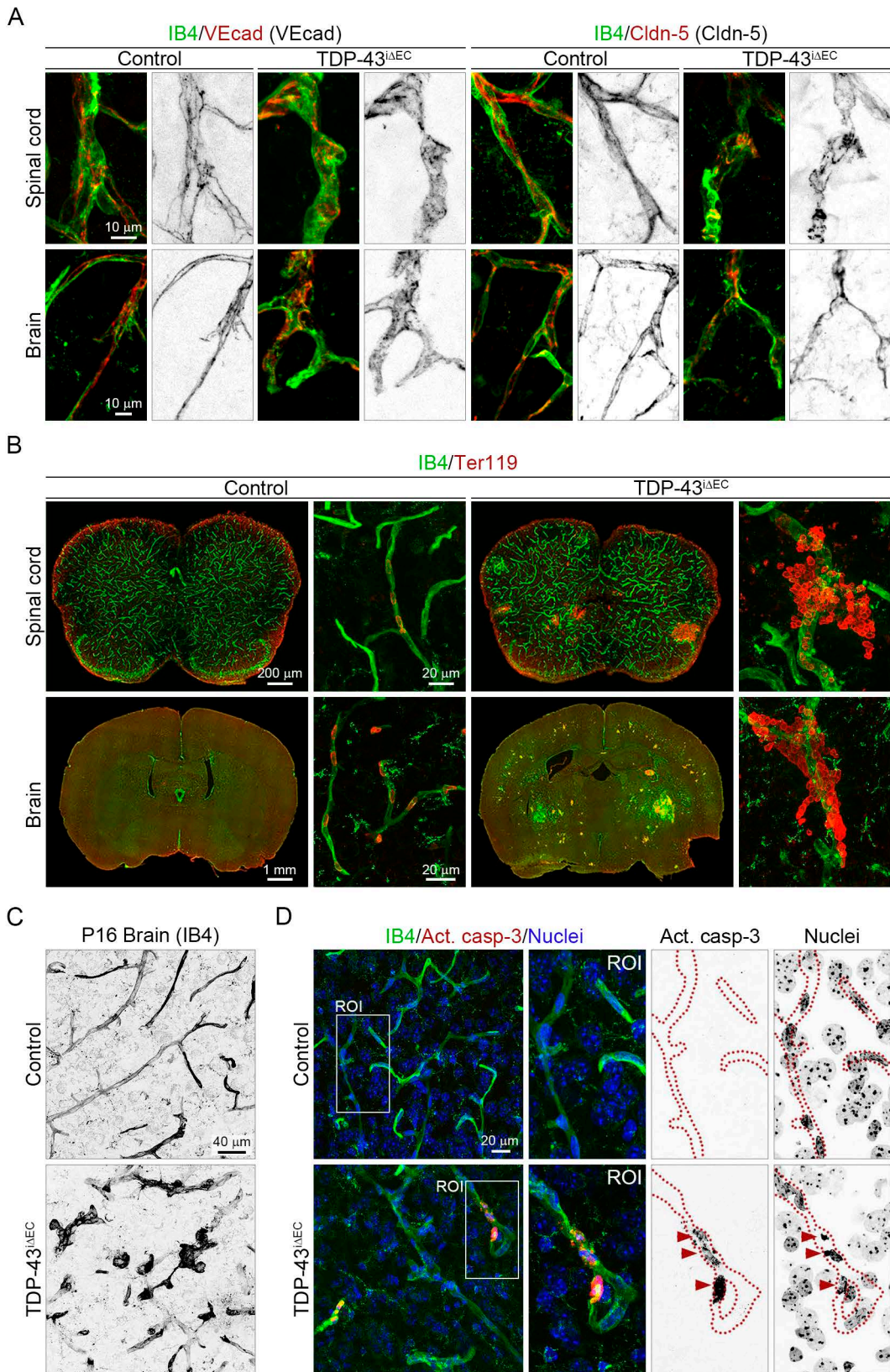
B



Supplemental Figure 3. Vascular malformation in *TDP-43^{iΔEC}* mice. (A) Confocal high-magnification images of P16 control and *TDP-43^{iΔEC}* retinas stained for IB4 (green) and Collagen-IV (red). White squares indicate the magnified ROIs. (B) Confocal high-magnification images of P16 control and *TDP-43^{iΔEC}* retinas stained for CD31 (green), Collagen-IV (red) and IB4 (blue).

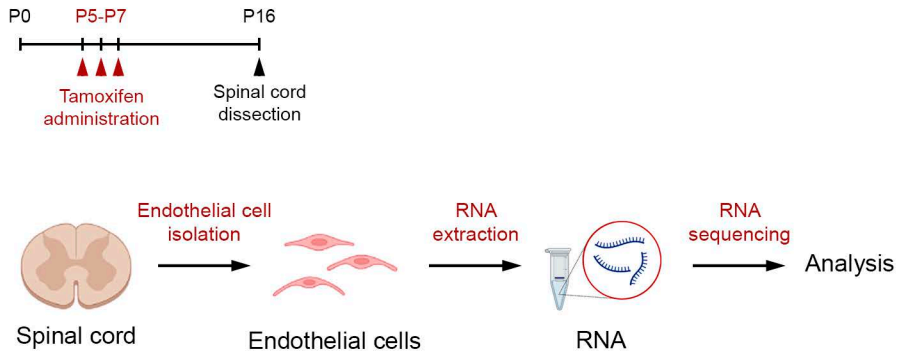


Supplemental Figure 4. Loss of TDP-43 disrupts the endothelial adherens junctions. (A) Confocal high magnification images of *TDP-43^{fl/fl}* and *TDP-43^{fl/fl};Cadh5^{Cre}* mLECs stained for VEcad (green), F-actin (red) and Hoechst (nuclei, blue). **(B)** Confocal high-magnification images of P16 control and *TDP-43^{iΔEC}* retinas stained for VEcad (green), F-actin (red) and IB4 (blue).

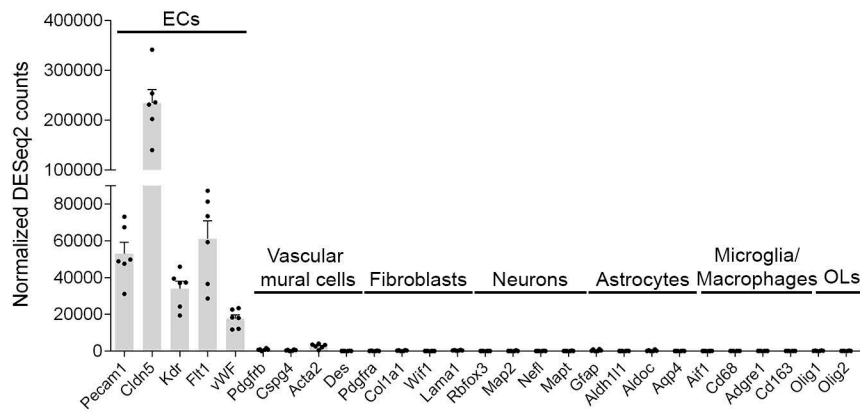


Supplemental Figure 5. Loss of TDP-43 disrupts the blood-CNS barrier and triggers vascular degeneration in the CNS. (A) Confocal high-magnification images of P16 control and *TDP-43^{iAEC}* spinal cord and brain sections stained for IB4 (green), VEcad (red) and Cldn-5 (red). (B) Confocal images of P16 control and *TDP-43^{iAEC}* spinal cord and brain sections stained for IB4 (green) and Ter119 (red), visualizing RBC leakage in *TDP-43^{iAEC}* vessels. (C) Confocal high-magnification images of P16 control and *TDP-43^{iAEC}* brain sections stained for IB4. Note the abnormal morphology of *TDP-43^{iAEC}* brain vessels. (D) Confocal high-magnification images of P16 control and *TDP-43^{iAEC}* brain sections stained for IB4 (green), Act. casp-3 (red) and Hoechst (nuclei, blue). White rectangles indicate magnified ROIs. Arrowheads indicate Act. casp-3 positive ECs. Dotted lines highlight the endothelial border of brain capillaries.

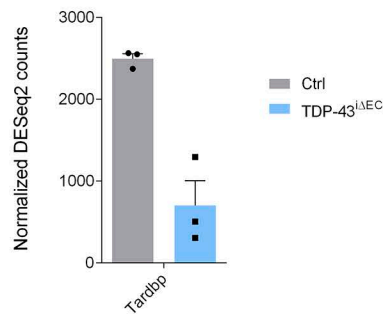
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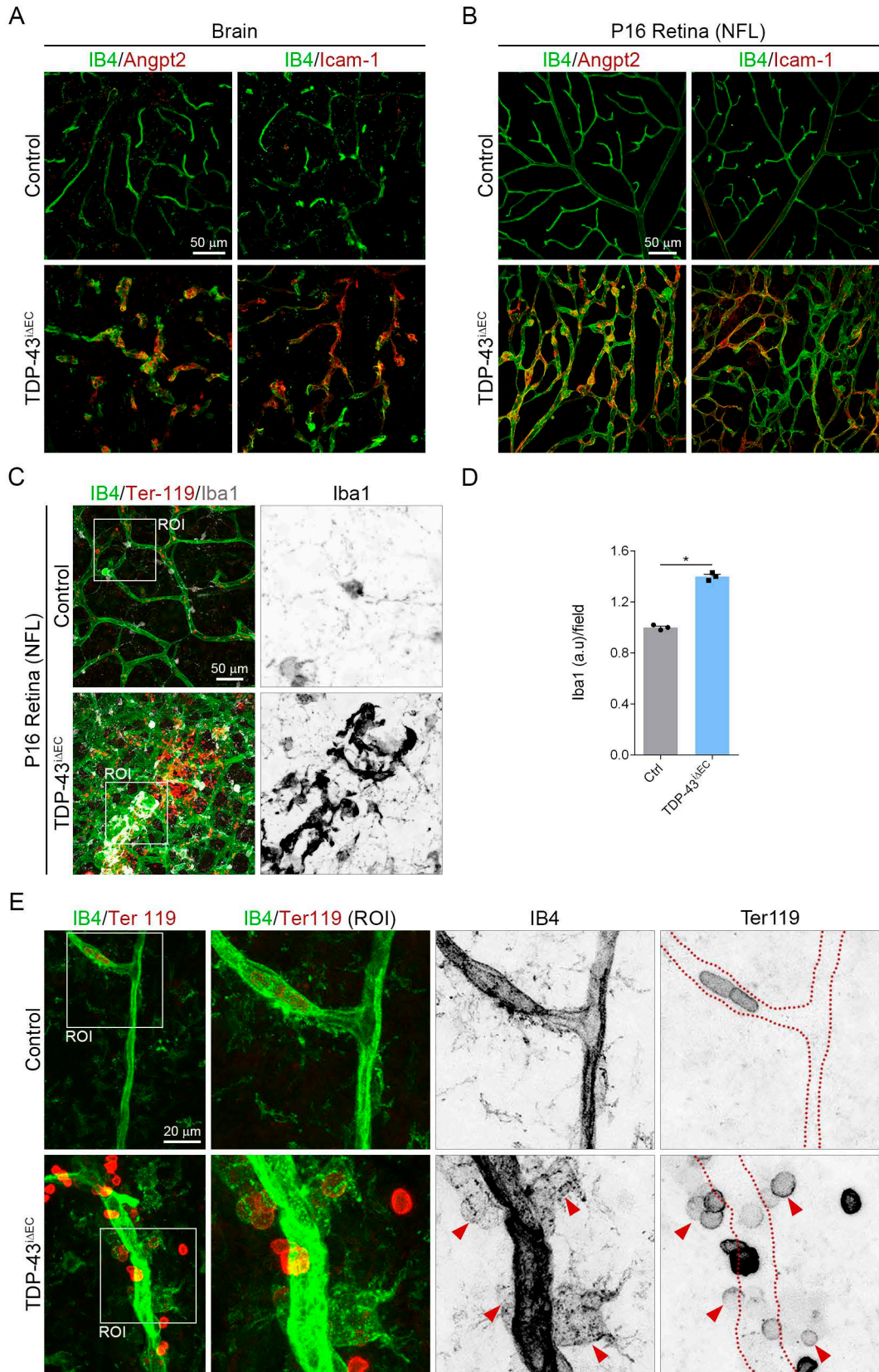
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C



Supplemental Figure 6. RNA sequencing analysis. (A) Diagram depicting the experiment schedule for ECs isolation and RNA sequencing analysis from spinal cords of P16 control and *TDP-43^{iΔEC}*. (B) Detailed comparison of expression levels for ECs, vascular mural cells, fibroblasts, neurons, astrocytes, microglia/macrophages and oligodendrocytes (OLs) canonical genes. (C) Detailed comparison of expression levels for *Tardbp* gene in ECs isolated from P16 control and *TDP-43^{iΔEC}* spinal cords.



Supplemental Figure 7. Inflammation in *TDP-43^{iAEC}* brain and retina. Confocal high-magnification images of IB4 (green), Angpt2 (red) and Icam-1 (red) in P16 brain (A) and retina (B). (C) Confocal high-magnification images of P16 control and *TDP-43^{iAEC}* retinas stained for IB4 (green), Ter119 (red) and Iba1 (white). White squares indicate the magnified ROIs. Note the enhanced Iba1 staining in *TDP-43^{iAEC}* samples and the morphological differences between Iba1+ cells from *TDP-43^{iAEC}* and control retinas. (D) Quantification of Iba1 fluorescence intensity per field in control and *TDP-43^{iAEC}* retinas as indicated. Error bars, s.e.m. p values (* $p < 0.05$). Mann-Whitney U test. (E) Confocal high-magnification images of P7 control and *TDP-43^{iAEC}* brain sections stained for IB4 (green) and Ter119 (red). White squares indicate the magnified ROIs. Note the presence of extravasated RBCs engulfed by IB4-positive macrophages (arrowheads) in *TDP-43^{iAEC}* mice. Dotted lines highlight the endothelial border of the blood vessels.

Supplemental Table 1. List of commercial antibodies and reagents.

Antigen/staining reagent	Source, number	Company	Dilution
β -Catenin (D10A8)	Rabbit, 8480	Cell Signaling	1:100
CD31 (MEC 13.3)	Rat, 553370	BD PharMingen™	-
Claudin 5	Rabbit, 34-1600	ThermoFisher Scientific	1:100
Claudin 5 (4C3C2)	Mouse, 35-2500	ThermoFisher Scientific	1:100
Cleaved caspase-3	Rabbit, 9661	Cell Signaling	1:200
Collagen-IV	Rabbit, 2150- 1470	Bio-Rad	1:200
Fibronectin	Rabbit, F3648	Sigma Aldrich	1:100
GAPDH	Mouse, MAB374	Chemicon	1:1000
GFAP	Rabbit, Z0334	Dako	1:250
Hoechst 33342	H3570	Invitrogen	1: 5000
Icam-1	Rabbit, ab222736	Abcam	1:100
Iba1	Rabbit, 019- 19741	Wako	1:1000
Ki67	Rabbit, ab16667	Abcam	1:100
mAngiopoietin-2	Sheep, AF7186	R&D Systems	1:100
Plvap	Rat, 550563	BD PharMingen™	1:100
TDP-43	Rabbit, 10782-2- AP	Proteintech	1:100 (IF mice), 1:500 (IF HUVECs), 1:5000 (WB)
Ter119	Rat, MAB1125	R&D Systems	1:100
α -Tubulin (DM1A)	Mouse, CP06	Calbiochem	1:1000
VE-cadherin (16B1)	Mouse, 14-1449- 82	ThermoFisher Scientific	1:100
VE-cadherin	Rat, 14-1442-85	Invitrogen	1:100
ERG Alexa Fluor-647	Rabbit, ab196149	Abcam	1:100
Paxillin	Mouse, 610051	BD PharMingen™	1:100
Phalloidin Alexa Fluor-633	A2284	Invitrogen	1:500 (IF HUVECs), 1:100 (IF mice)
Isolectin-B4 Alexa Fluor-488	I21411	Invitrogen	1:150
Mouse Alexa Fluor-546	Donkey, A10036	Invitrogen	1: 500
Rabbit Alexa Fluor-488	Chicken, A21441	Invitrogen	1: 500
Rabbit Alexa Fluor-546	Donkey, A10040	Invitrogen	1:500

Rabbit Alexa Fluor-633	Goat, A21070	Invitrogen	1:500
Rat Alexa Fluor-546	Goat, A11081	Invitrogen	1:500
Rat Alexa Fluor-633	Goat, A21094	Invitrogen	1:500
Sheep Alexa Fluor-546	Donkey, A21098	Invitrogen	1:500
APC Rat IgG2a κ Isotype Control	553932	BD Pharmlngen™	1:1000
APC Rat Anti-mouse CD31 (Clone: MEC 13.3)	561815	BD Pharmlngen™	1:1000
PE-Cy™7 Mouse IgG1 κ Isotype Control	557646	BD Pharmlngen™	1:1000
PE-Cy™7 Rat Anti-mouse CD45 (Clone: 30-F11)	552848	BD Pharmlngen™	1:1000
PE-Cy™7 Rat Anti-mouse TER- 119/Erythroid cells	557853	BD Pharmlngen™	1:1000