

Natural light experience induces metabolic switch that promotes cerebrovascular maturation

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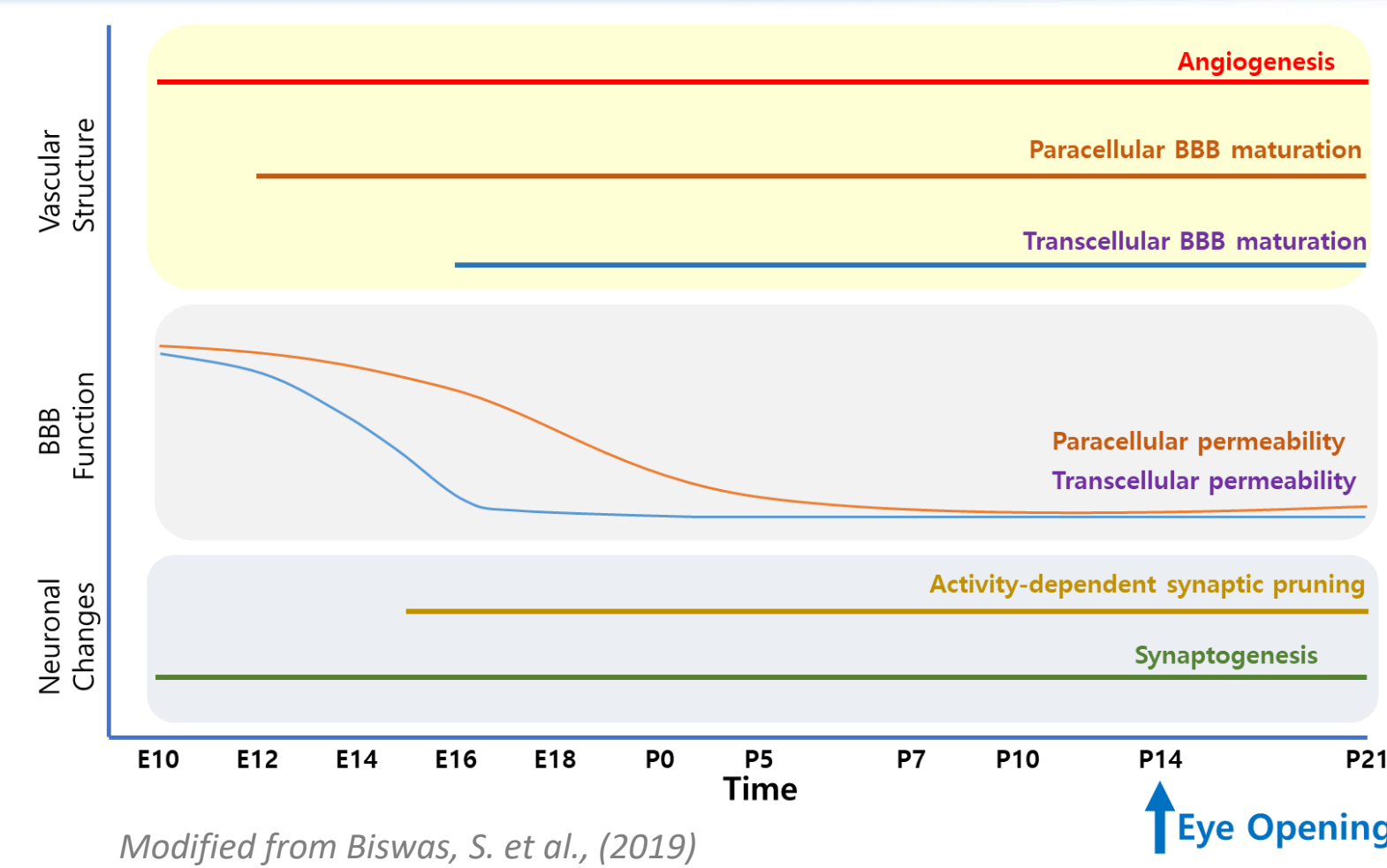
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Abstract

Early light exposure is a key environmental factor that accelerates brain development in mice. When their eyes open around postnatal day 14 (P14), light triggers molecular and structural changes in the brain. These changes affect neuronal activity, influencing angiogenesis, blood flow, and blood-brain barrier (BBB) function. The vascular responses to these changes are complex and depend on various factors, including the specific neurons activated, the brain region involved, and the age of the animal. However, *much less is known about the underlying molecular mechanism in brain endothelial cells (BECs) following sensory experience during development*. In this study, we employed translatome analysis with the RiboTag method to compare BECs at P10 and P30, before and after eye opening, and identified 32 genes that respond specifically to light, through a parallel comparison with a previously reported database. We confirmed that light exposure induces developmental changes in the expression of light-responsive genes, with these changes completely abolished in dark-reared mice. Among these genes, *the expression of monocarboxylate transporter 1 (MCT1), a lactate transporter, showed a significant decrease in response to light, whereas glucose transporter 1 (GLUT1) expression increased*. Under dark-reared conditions, this metabolic shift was abolished, accompanied by vascular leakage and immature astrocytes in the visual cortex. Moreover, we found that *the activation of TGF- β signaling played a role in the reciprocal switch of both metabolic transporters in BECs*. In conclusion, the neuronal activation may suppress TGF- β signaling, possibly via astrocytes, thereby modulating the balance of lactate and glucose metabolism through the regulation of MCT1 and GLUT1 expression in BECs. This process ultimately contributes to vascular maturation, including the BBB formation.

Background

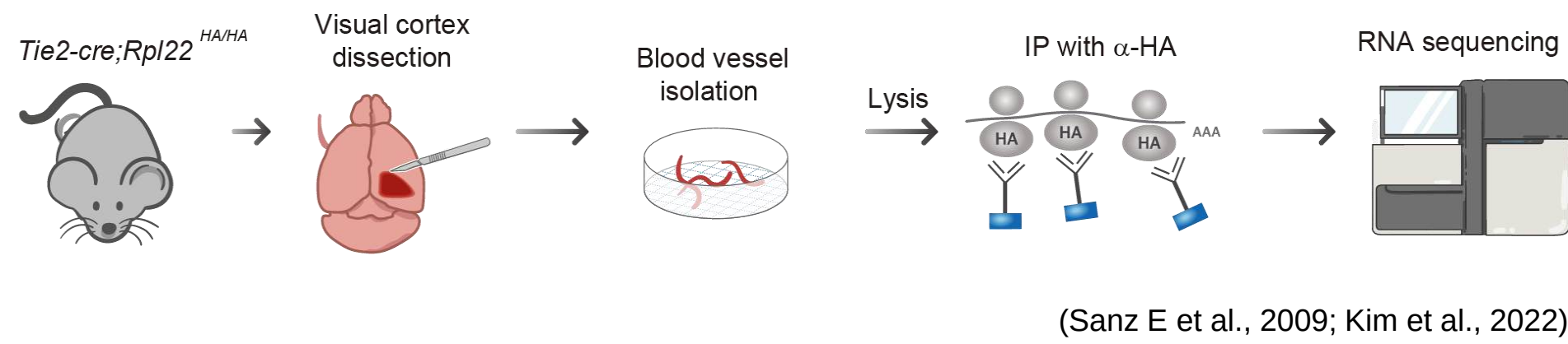


Questions

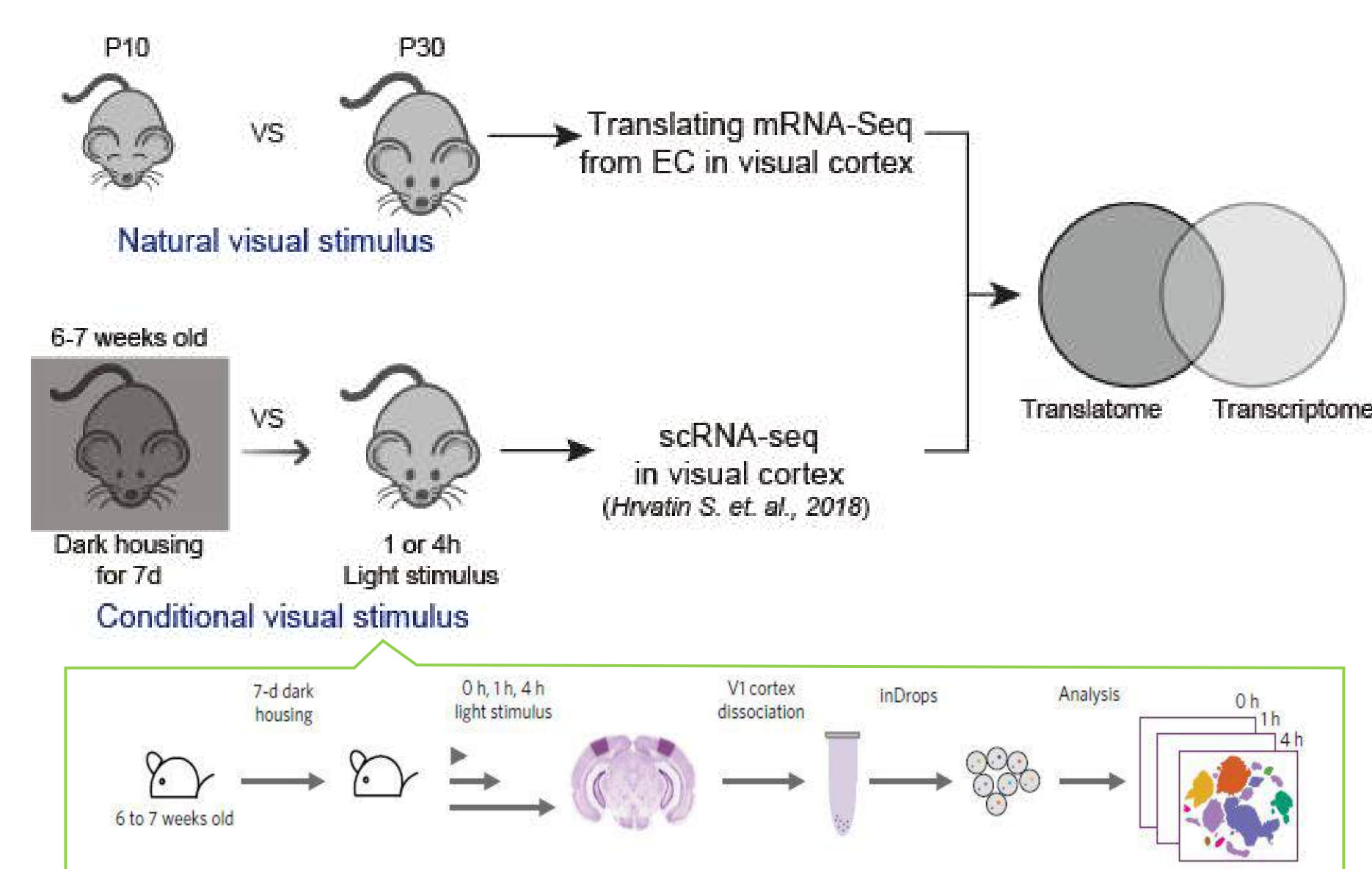
To understand the molecular mechanisms in BECs following sensory experience during development

Methods

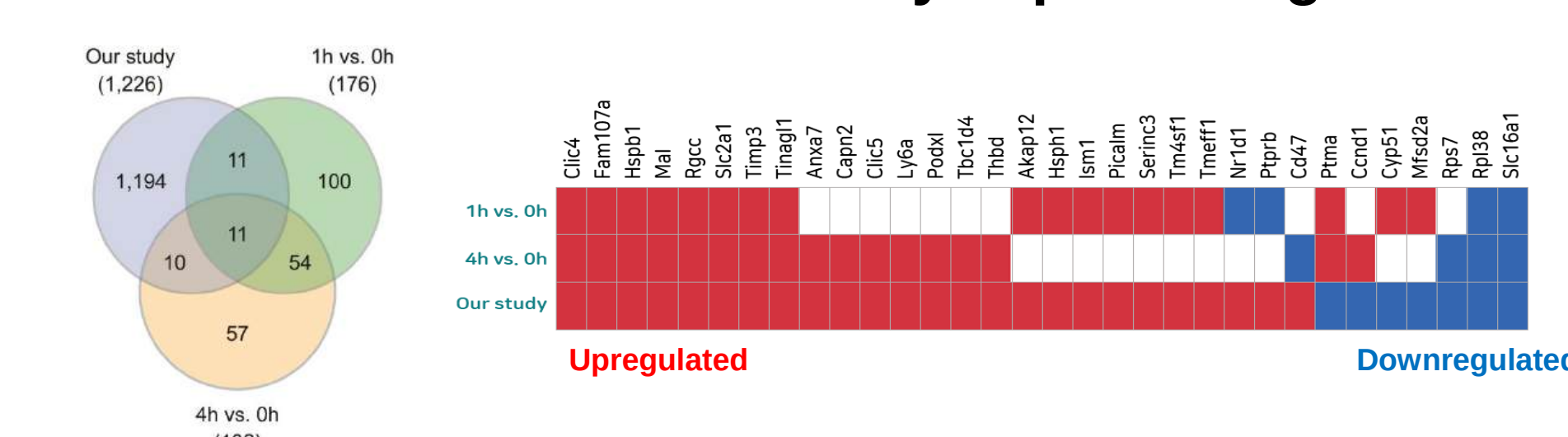
Brain EC-specific Translatome using RiboTag Mouse



Neuronal stimulation models by light



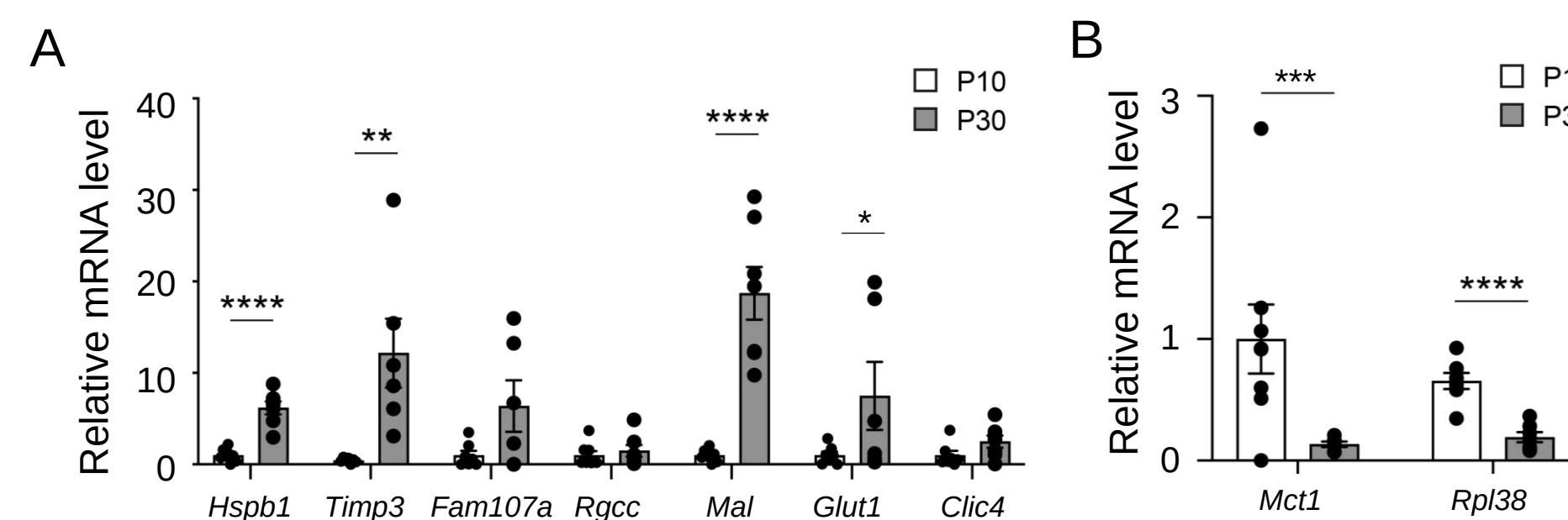
Identification of differentially expressed genes



Bioinformatic analysis identified 32 genes that overlapped with our translatome and previously published datasets (Havatin et al., 2018, *Nat Neurosci.*).

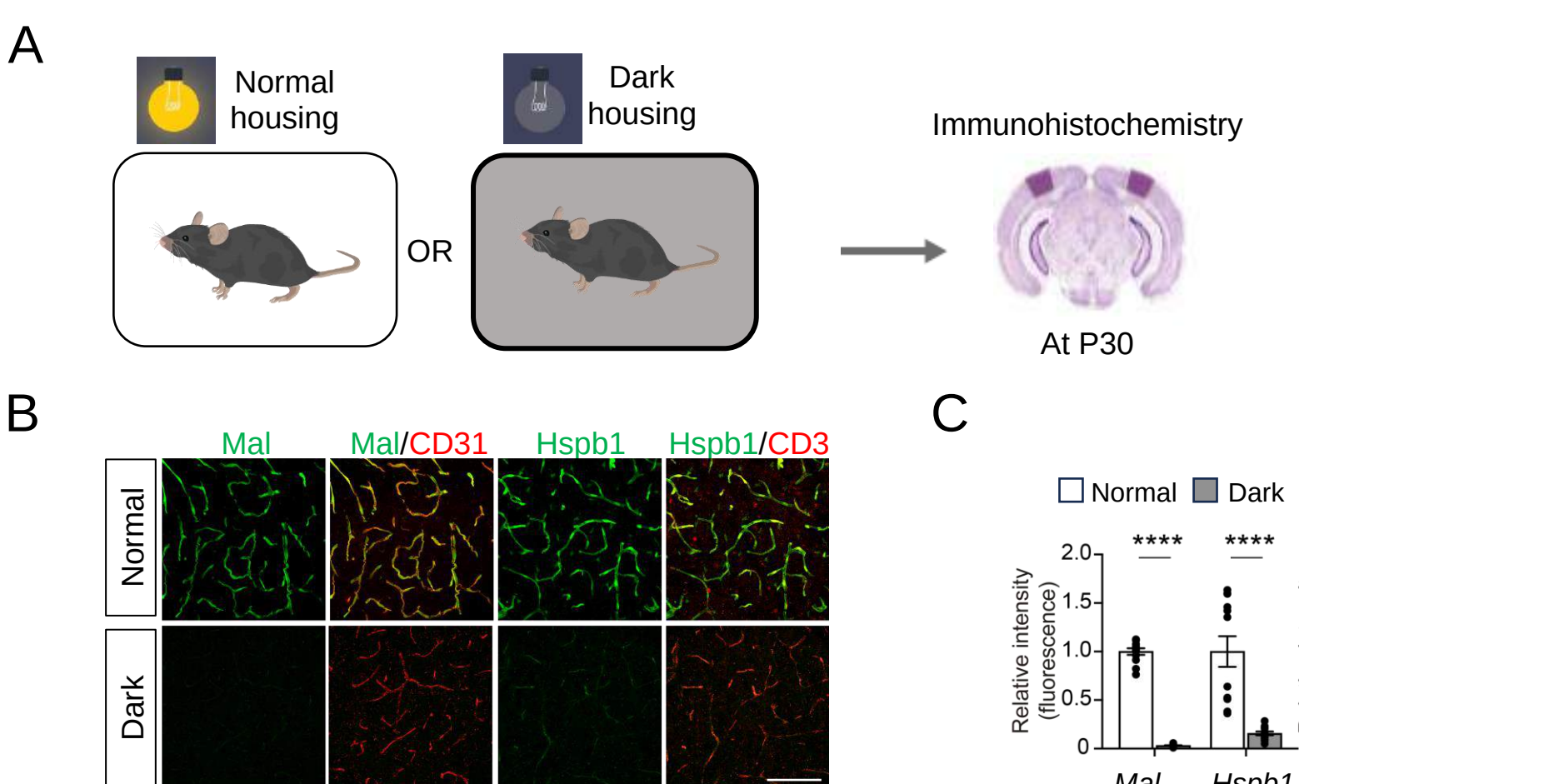
Result I

Verification of the target genes in the visual cortex



Validation of upregulated genes (A) and downregulated genes (B) by RT-qPCR.

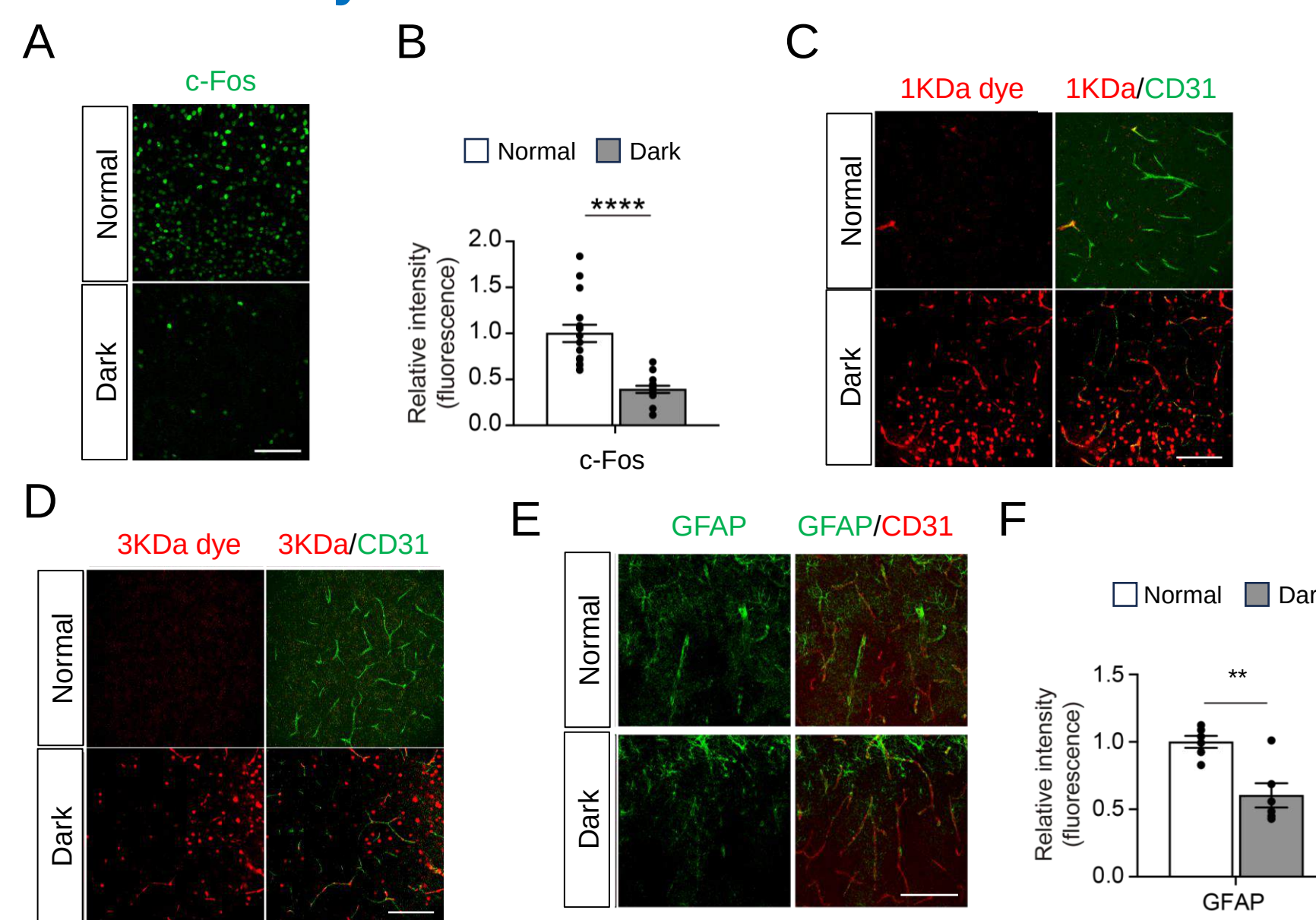
Changes in expression of target genes were completely abolished in the absence of light



(A) Schematic diagram illustrating workflow (B) Representative images showing the expression of *Mal* and *Hspb1* (green) in the BEC (red) of the visual cortex in mice reared in normal or dark cages. Scale bar, 100 μm. (C) Quantification of fluorescence intensity in (B).

Result II

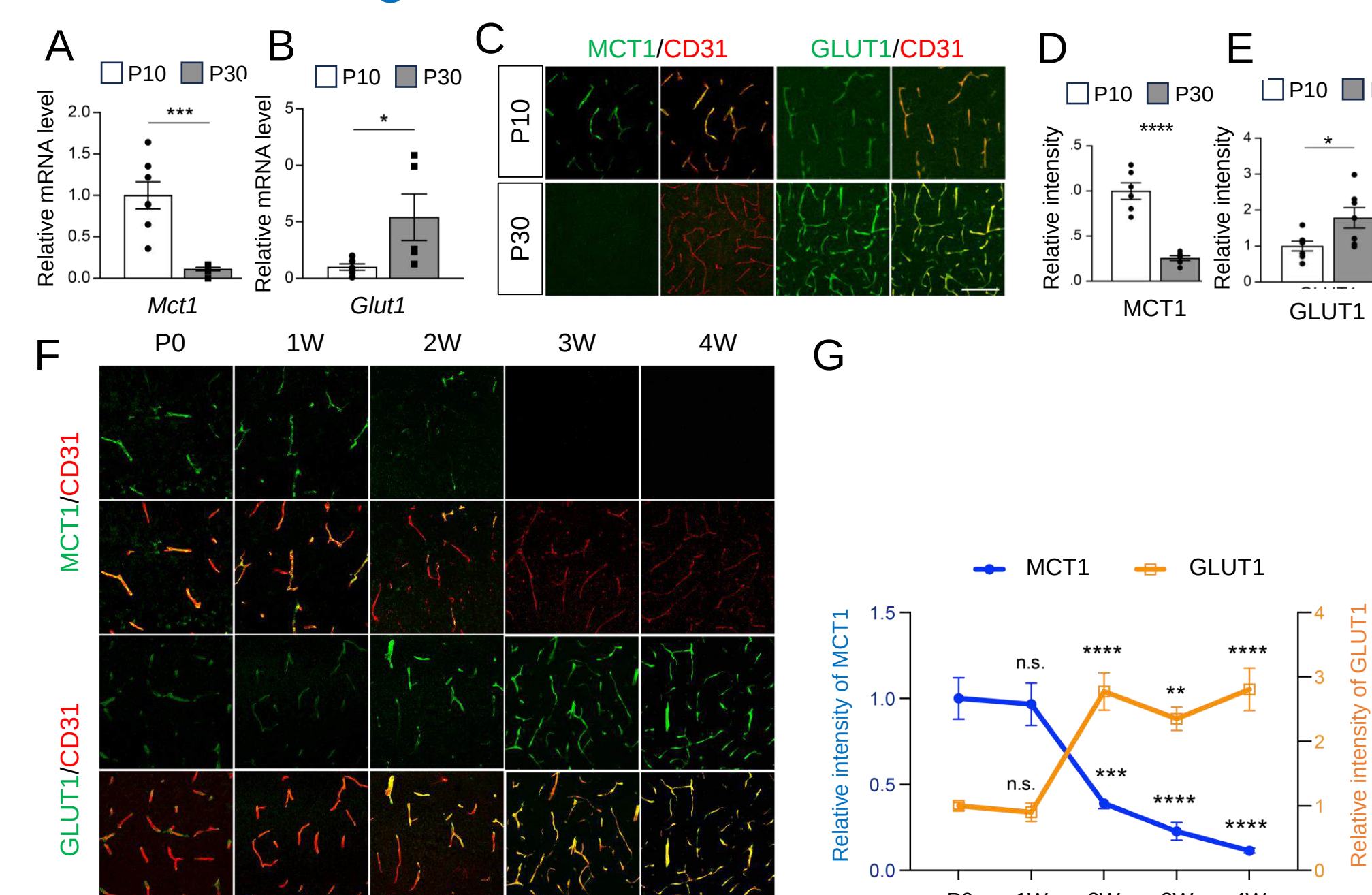
Dark rearing impaired the development of the BBB and astrocytes



(A-B) Representative images and quantification of c-Fos (green) in the visual cortex of control or dark-reared mice. Scale bar, 100 μm. Dark rearing induced the vascular leakage in the visual cortex. (Alexa Fluor™ 555 Cadaverine ; red) in (C) and (Dextran-Texas Red™, 3000 MW, Lysine Fixable; red) in (D). Scale bars, 100 μm. (E) Immunohistochemistry of GFAP (green) and CD31 (red) showing immature astrocytes in the visual cortex after light deprivation. Scale bar, 100 μm. (F) Quantification of fluorescence intensity in (E).

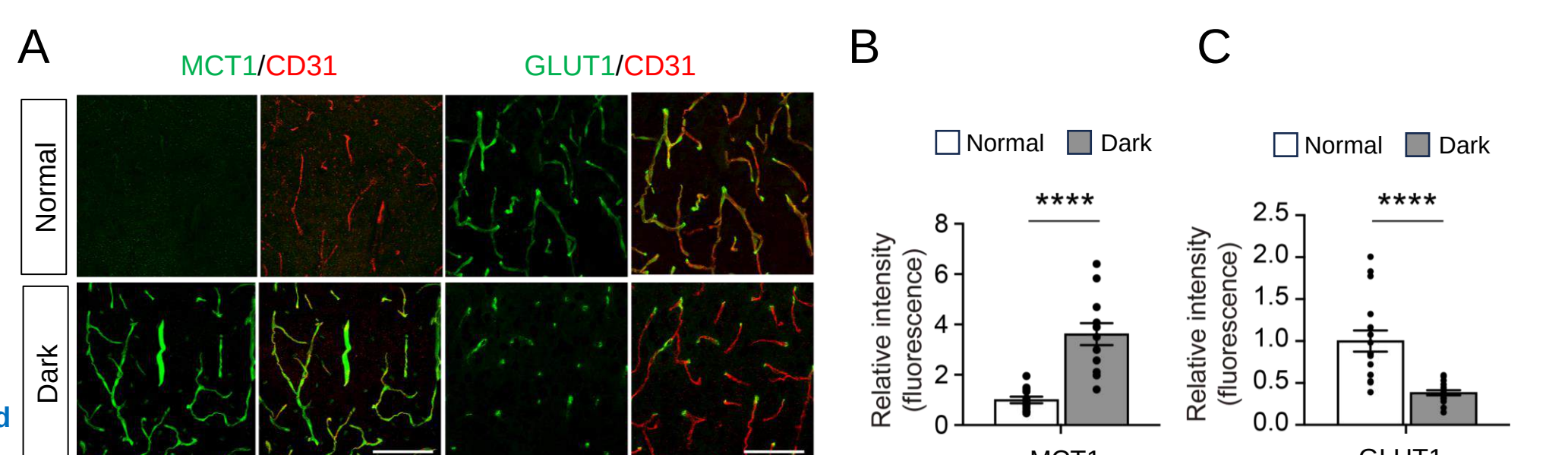
Result III

Exposure to light decreases MCT1 expression while inducing an increase in GLUT1



RT-qPCR (A-B) and immunohistochemistry (C). Scale bar, 100 μm. (D-E) Quantification of fluorescence intensity in (C). (F) Representative images of MCT1 (green) or GLUT1 (green) in endothelial cell (CD31, red) of the visual cortex with aging. Scale bar, 100 μm. (G) Quantification of fluorescence intensity in (F).

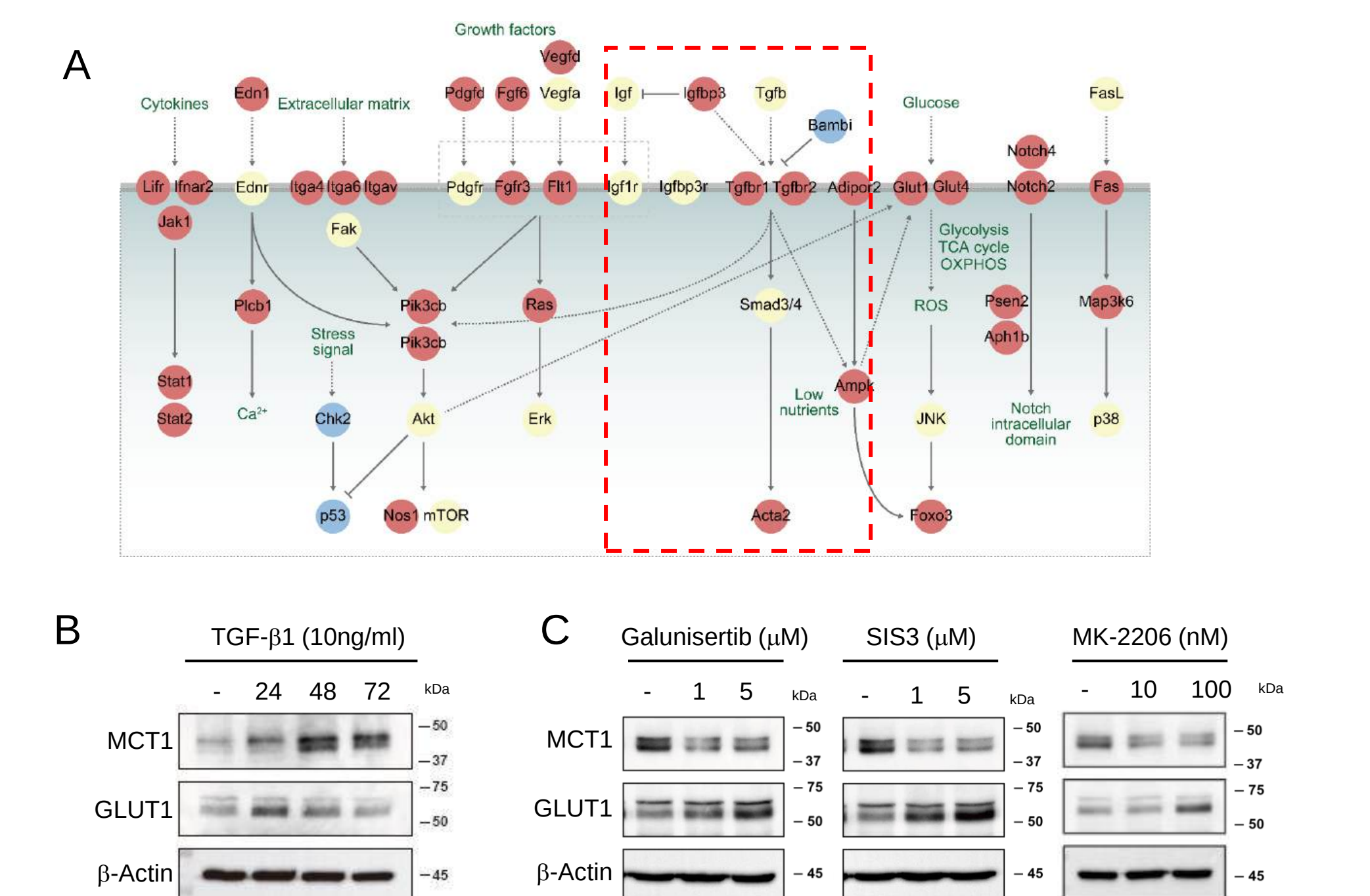
Under dark rearing conditions, the expression patterns of MCT1 and GLUT1 are reversed



(A) Representative images showing the expression of MCT1 and GLUT1 (green) in the BEC (red) of the visual cortex in mice reared in normal or dark cages. Scale bars, 100 μm. (B-C) Quantification of fluorescence intensity in (A).

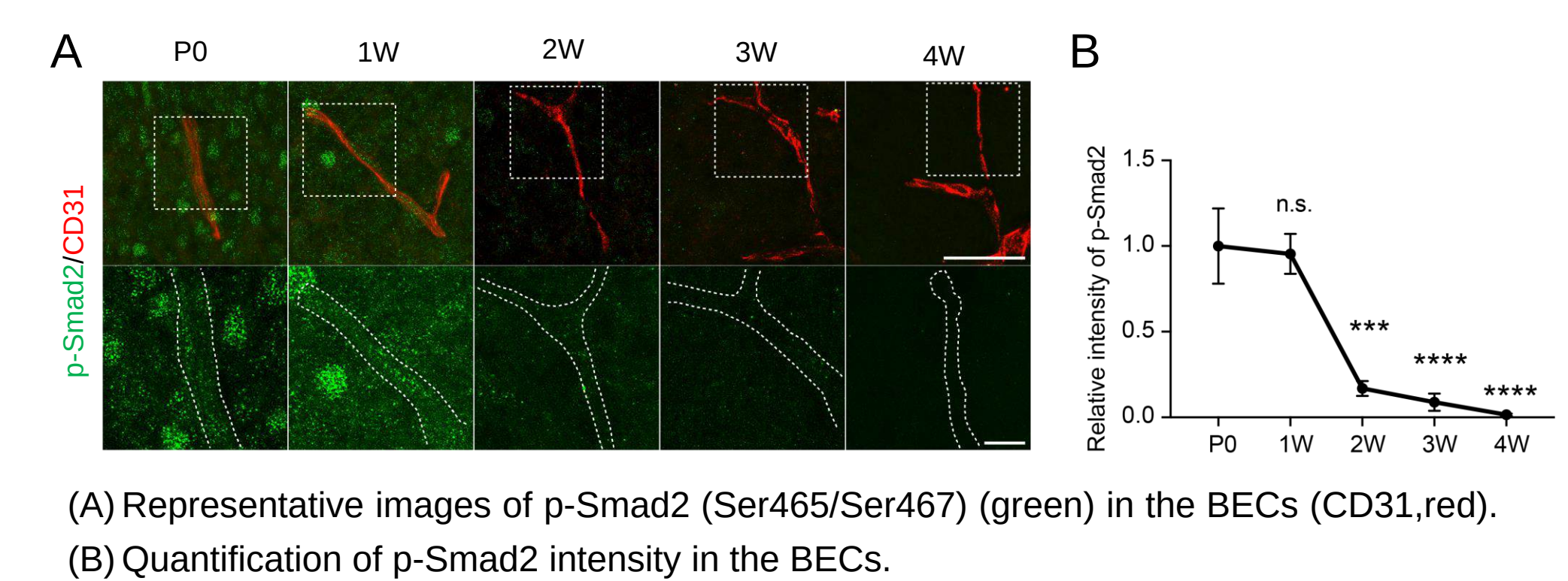
Result IV

TGF- β signaling is involved in the shift from lactate to glucose transporters in BEC during development



(A) Gene expression network analysis. (B) Western blot data showing that TGF- β treatment increases MCT1 expression and inhibits GLUT1 expression in hCMEC/D3 cells. (C) Western blot images showing MCT1 and GLUT1 expression in hCMEC/D3 cells after treatment by Galunisertib (TGF- β receptor 1 inhibitor), SIS3 (Smad3 inhibitor), and MK-2206 (AKT inhibitor).

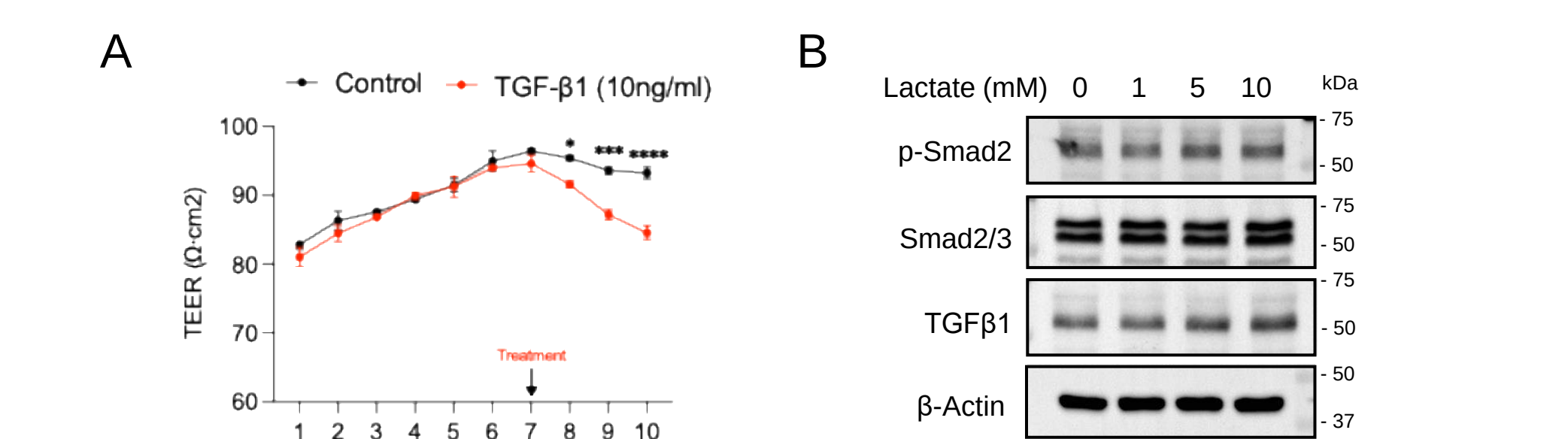
TGF- β signaling declines in the BECs of developing visual cortex



(A) Representative images of p-Smad2 (Ser465/Ser467) (green) in the BECs (CD31, red). (B) Quantification of p-Smad2 intensity in the BECs.

Result V

TGF- β 1 signaling increases monolayer permeability and is induced by lactate treatment

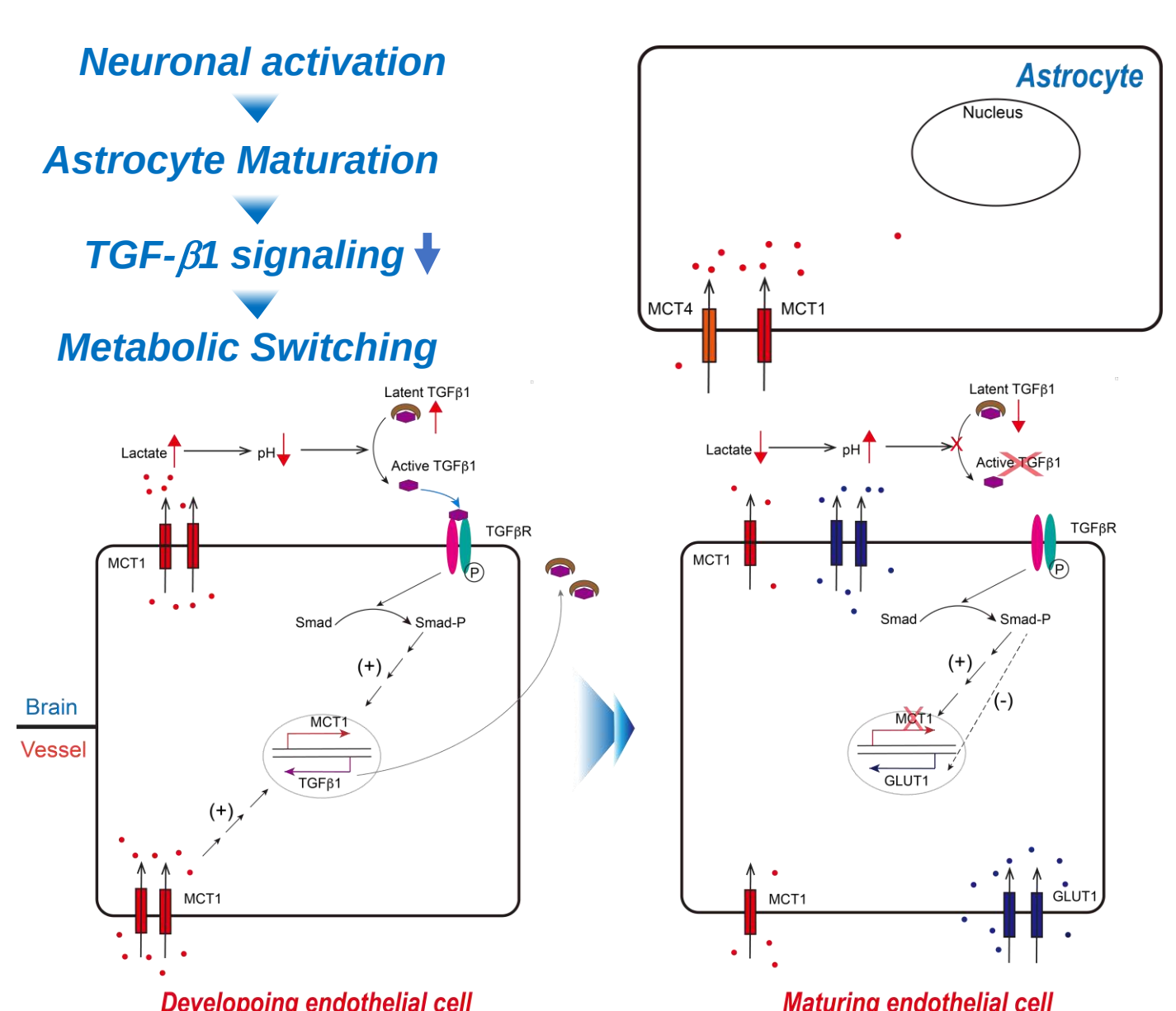


(A) Monolayer permeability measurement using TEER (TransEndothelial Electrical Resistance) with EVOM2. (B) Western blot analysis of p-Smad (Ser465/Ser467) and TGF- β 1 ligand following lactate treatment in hCMEC/D3 cells.

Current conclusion

- ✓ Light-induced neuronal activation alters the metabolic regulation of brain endothelial cells by modulating the expression of MCT1 and GLUT1 in opposing directions
- ✓ TGF- β signaling may be involved in the pathway that regulates the opposing modulation of MCT1 and GLUT1 expression

Hypothesis



Acknowledgement

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