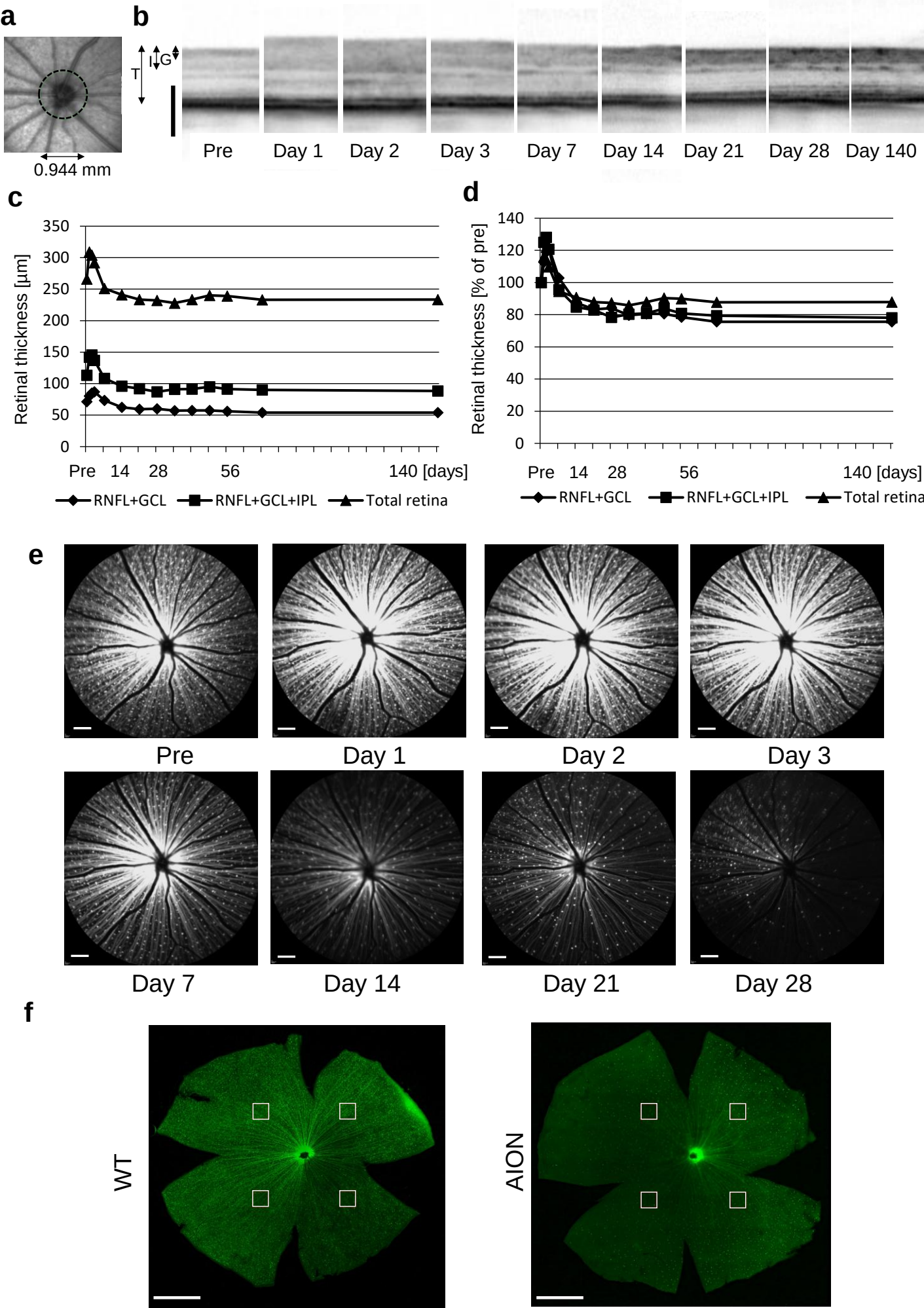


Supplementary Figures

Valosin-containing protein modulator KUS121 protects retinal neurons in a rat model of anterior ischemic optic neuropathy

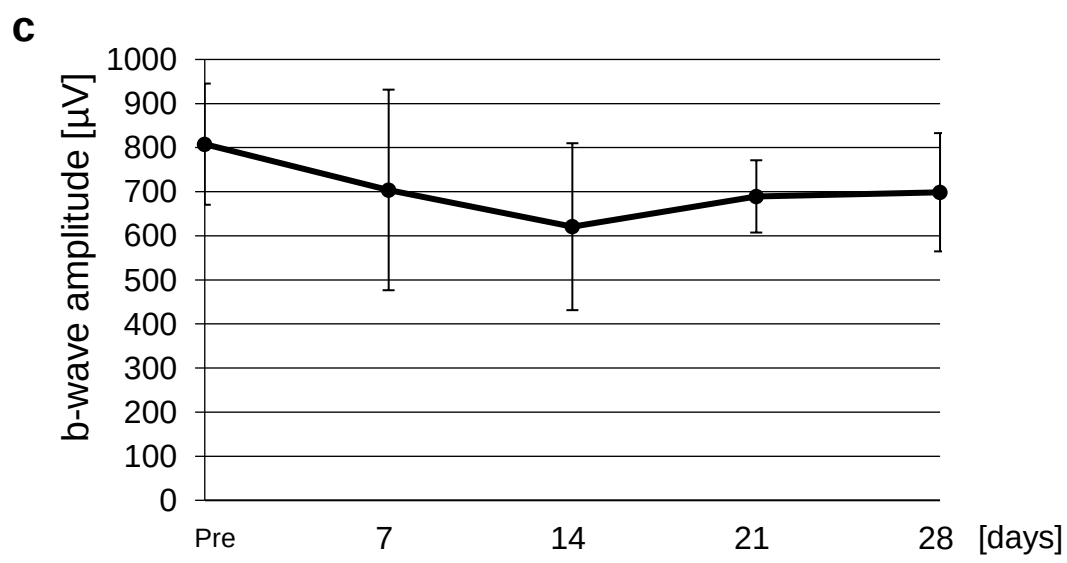
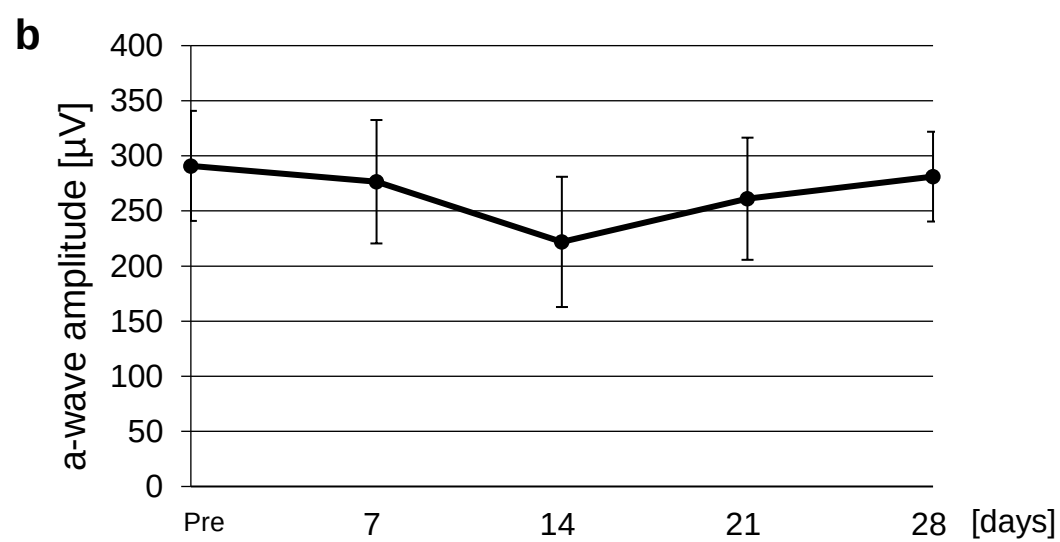
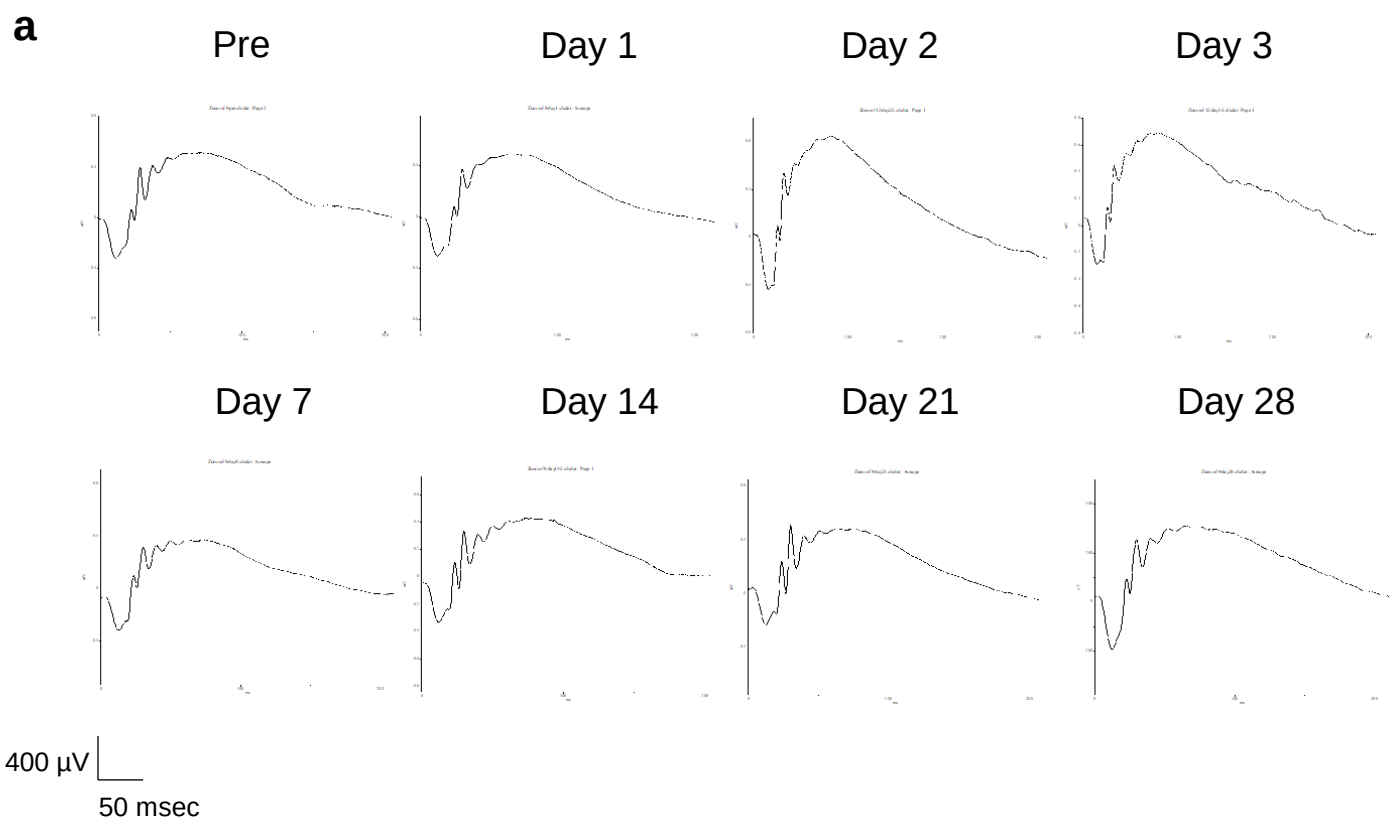
Chinami Kikkawa, Hanako O. Ikeda, Masayuki Hata, Sachiko Iwai, and Akitaka Tsujikawa

Supplementary Figure S1



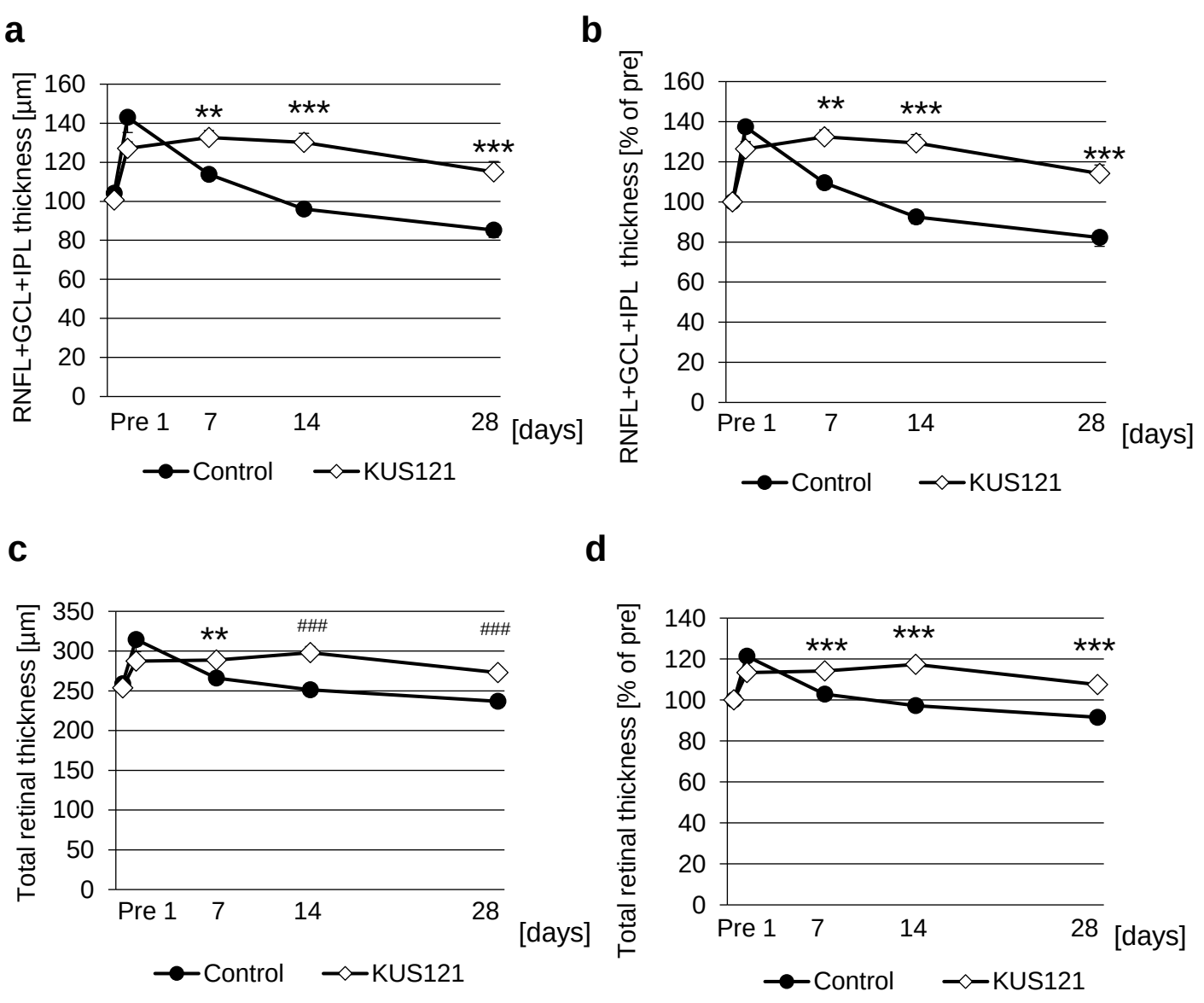
Supplementary Fig. S1. Time-related changes in the retina of a rat model of anterior ischemic optic neuropathy (AION). **(a)** Fundus scanning laser ophthalmoscopy image shows a dotted circle indicating the peripapillary scan region used for optical coherence tomography (OCT). In rats, this corresponds to a diameter of 0.944 mm. **(b)** Representative OCT images of rat retina before and at various time points up to 140 days post-AION induction (days 1, 2, 3, 7, 14, 21, 28, and 140). The bidirectional arrows indicate the total retina (T), the retinal nerve fiber layer (RNFL) + ganglion cell layers (GCL) + inner plexiform layer (IPL) (I, inner retinal thickness), and RNFL + GCL (G). **(c, d)** Temporal changes in the thickness of the total retina, RNFL + GCL + IPL, and RNFL + GCL (c) and their percentage change from the baseline (d) at 1 to 140 days post-AION induction (n = 2). Error bars represent standard error (SE). **(e)** Representative fluorescein fundus photography images before and 1 to 28 days post-AION induction. **(f)** Retinal flat mounts of wild-type (WT) and AION-induced rats at day 28. The white squares indicate the region where retinal ganglion cell (RGC) counts were measured (within four 500- μ m squares located 2000 μ m from the optic nerve head center). Scale bars: 200 μ m in (b), 500 μ m in (e), 2000 μ m in (f).

Supplementary Figure S2



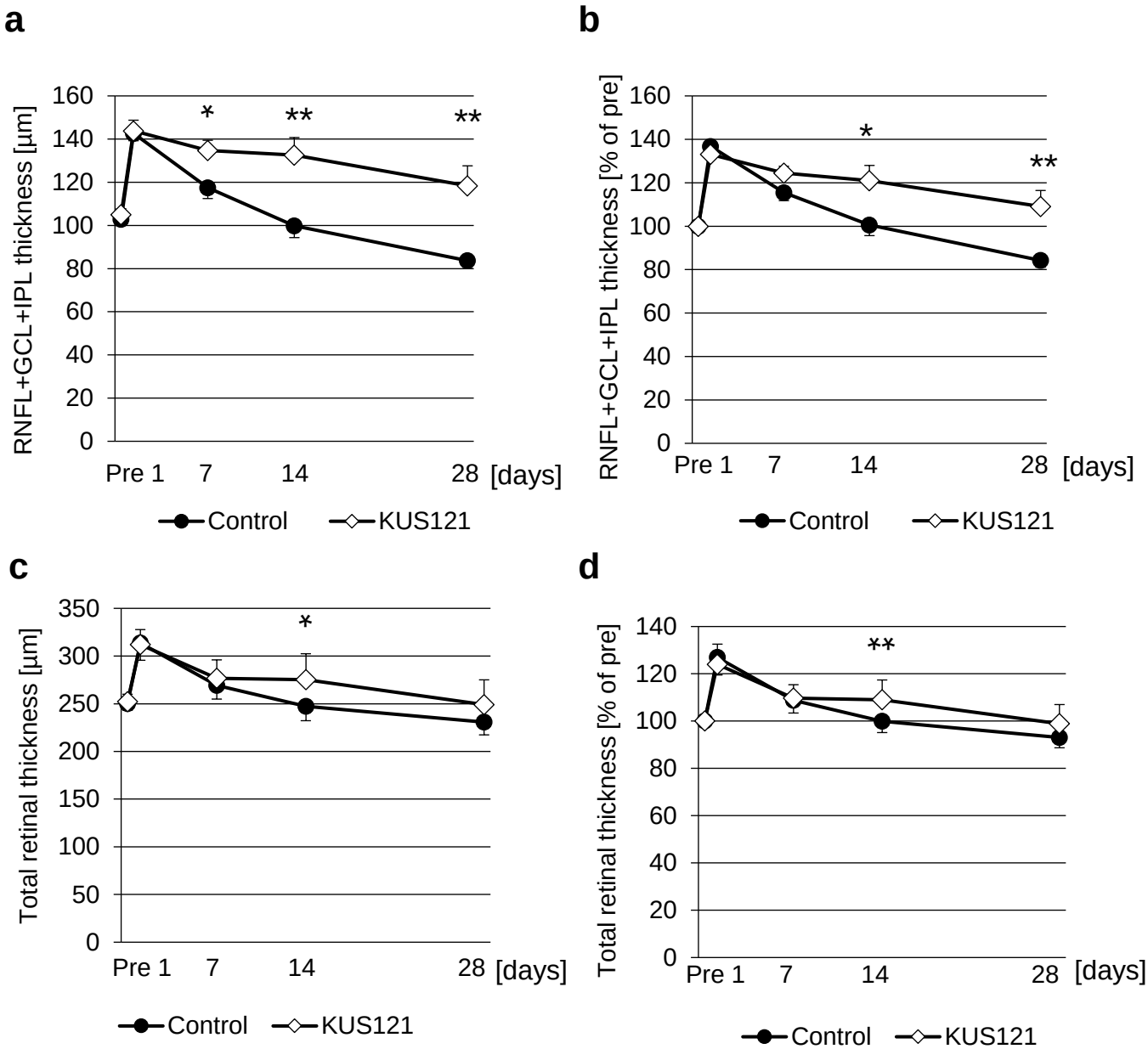
Supplementary Fig. S2. Time-related changes in electroretinography in a rat model of anterior ischemic optic neuropathy (AION). (a) Representative scotopic electroretinography images with intensities of $3 \text{ cd} \cdot \text{s} \cdot \text{m}^{-2}$ taken before, and 1, 2, 3, 7, 14, 21, and 28 days post-AION induction. **(b, c)** Temporal changes in a-wave (b) and b-wave (c) amplitudes. $n = 6$, error bars represent SE.

Supplementary Figure S3



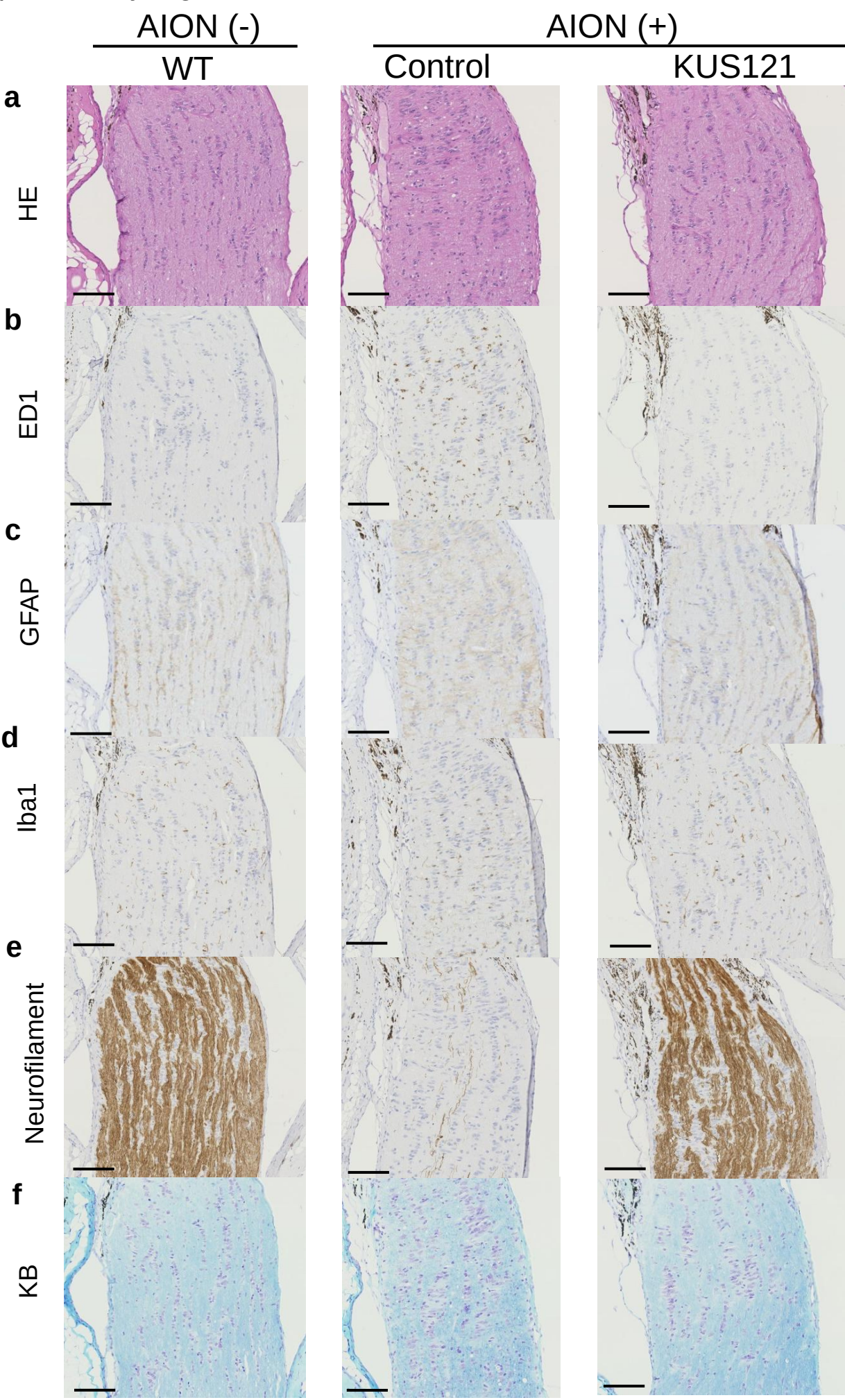
Supplementary Fig. S3. Time-related retinal thickness changes in rats with anterior ischemic optic neuropathy (AION) following pre-intravitreal injection with KUS121. (a, b) Inner retinal thickness (RNFL + GCL + IPL) (a) and percentage change compared to pre-treatment data (b) rats with AION intravitreally injected with KUS121 (n = 10, squares) or vehicle (control) (n = 8, circles). See Figure 1a. (c, d) Total retinal thickness (c) and percentage change compared to pre-treatment data (d). Error bars indicate SE. **P < 0.01, ***P < 0.001 vs. control (Student's t-test), ####P < 0.001 vs. control (Aspin Welch's t-test).

Supplementary Figure S4



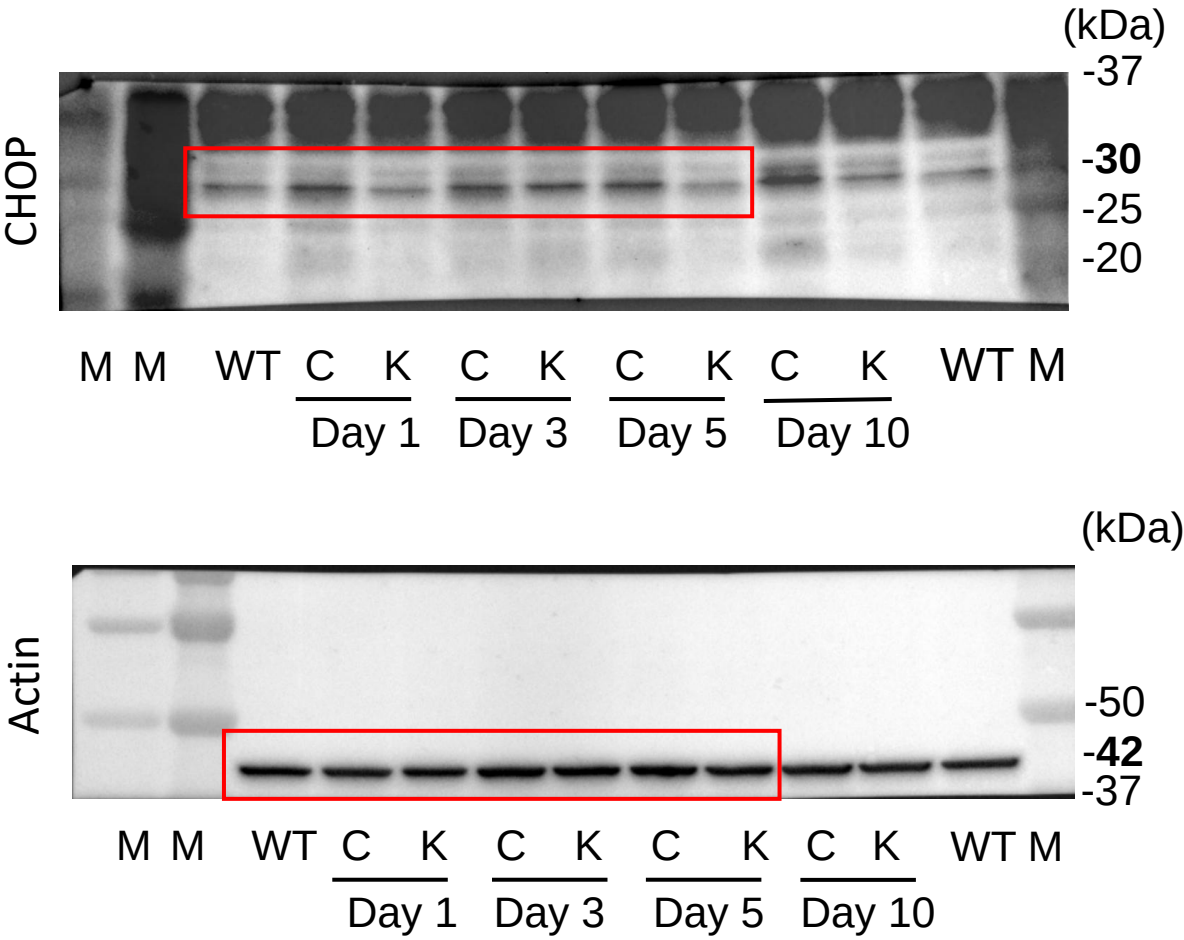
Supplementary Fig. S4. Time-related changes in retinal thickness in rats with anterior ischemic optic neuropathy (AION) following intravitreal injection of KUS121 post-AION induction. (a, b) Inner retinal thickness (RNFL + GCL + IPL) (a) and percentage change compared to pre-treatment data (b) in AION rats intravitreally injected with KUS121 (n = 8, squares) or vehicle (control) (n = 8, circles). See Figure 2a. (c, d) Total retinal thickness (c) and percentage change compared to pre-treatment data (d). Error bars indicate SE. *P < 0.05, **P < 0.01 vs. control (Student's t-test).

Supplementary Figure S5



Supplementary Fig. S5. Low-magnification images of Figure 3a–f. Histological examination of optic nerve sections in wild-type (WT), vehicle-treated (control), and KUS121-treated (KUS121) rats 28 days post-induction of anterior ischemic optic neuropathy (AION). Images of sections stained with hematoxylin-eosin (HE) (a), and immunostained with anti-macrophage (ED1) (b), glial fibrillary acidic protein (GFAP) (c), ionized calcium-binding adapter molecule 1 (Iba1) (d), neurofilament (e), and Klüver-Barrera (KB) staining (f). Cells were counterstained with hematoxylin or cresyl violet. Bar = 100 μ m.

Supplementary Figure S6



Supplementary Fig. S6. Full-size images of gel blots related to Figure 5a.

To quantify CHOP and actin expression levels, the membrane was cut at 37 kilodalton (kDa) after blotting, with one portion used for the CHOP antibody and the other for actin. M: molecular marker, C: AION-induced rat, K: AION-induced rat treated with KUS121, WT: wild-type rat.