Supplementary information

Granulin 1 Promotes Retinal Regeneration in Zebrafish

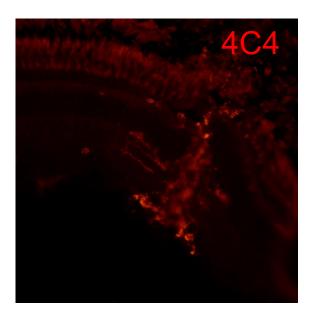
Kazuhiro Tsuruma^{1,2}, Yuichi Saito¹, Hiroyuki Okuyoshi¹, Akihiro Yamaguchi¹, Masamitsu Shimazawa¹, Daniel Goldman², and Hideaki Hara^{1*}

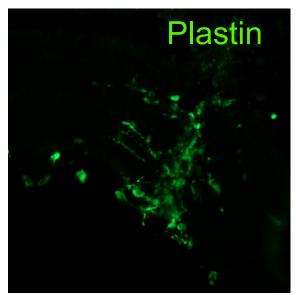
¹Department of Biofunctional Evaluation, Molecular Pharmacology, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu 501-1196, Japan. ²Molecular and Behavioral Neuroscience Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109, USA.

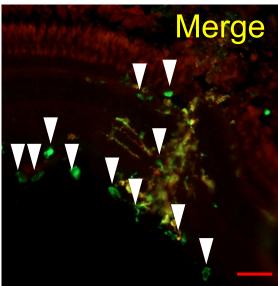
*Corresponding author: Prof. Hideaki Hara, Ph.D., R.Ph.

Address: Department of Biofunctional Evaluation, Molecular Pharmacology, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu 501-1196, Japan.

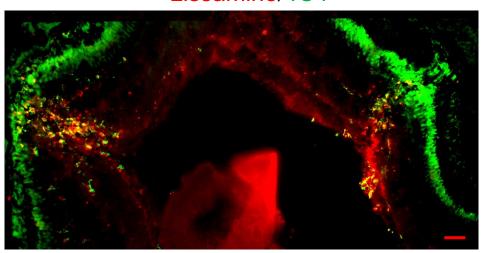
Tel./Fax: +81-58-230-8126; E-mail: hidehara@gifu-pu.ac.jp

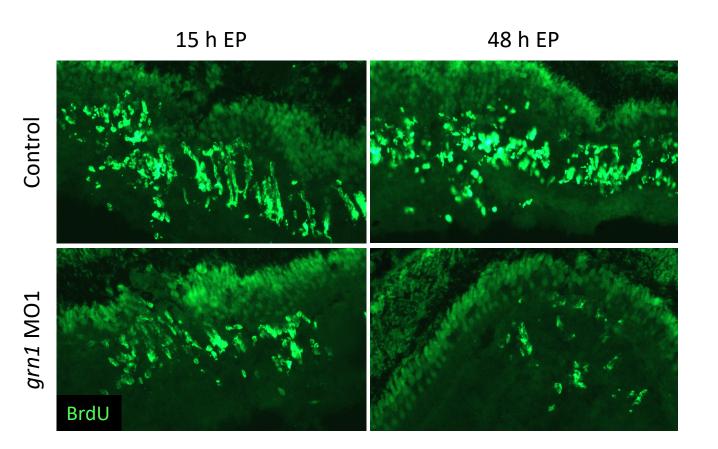




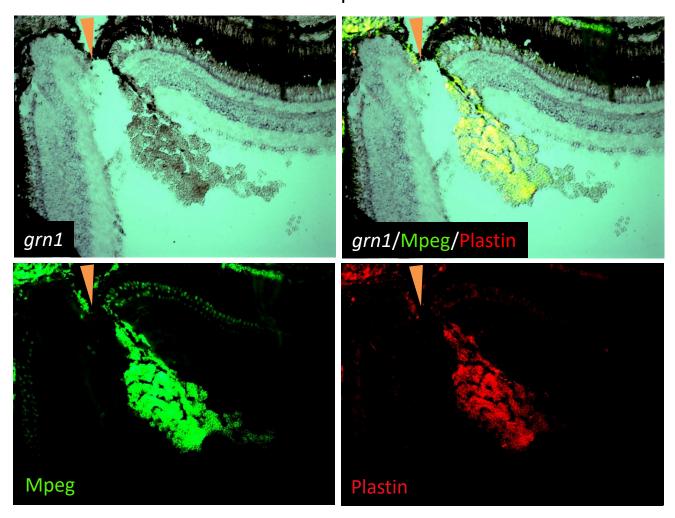


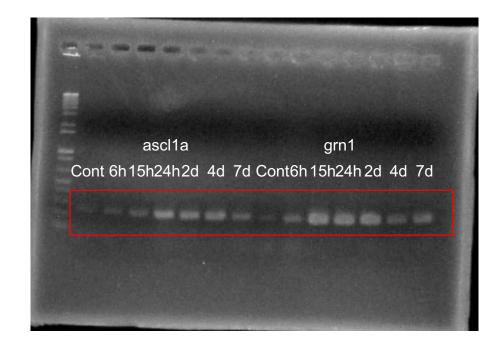
Lissamine/4C4

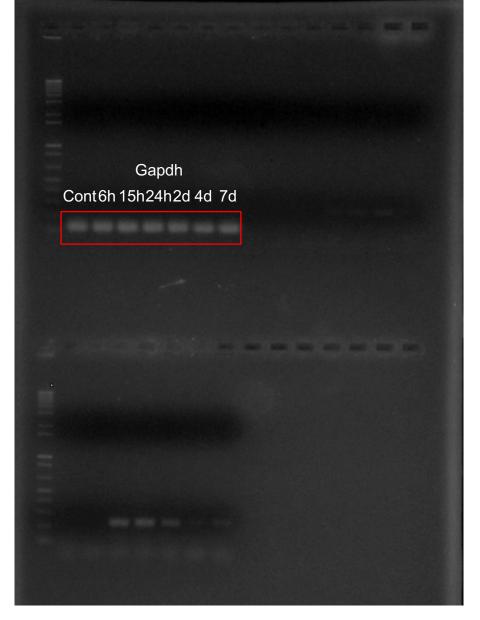




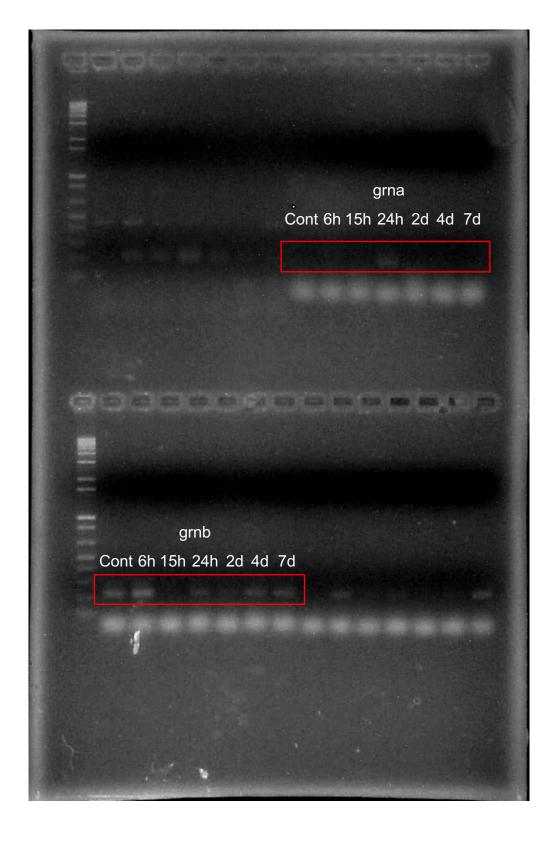
3 hpi

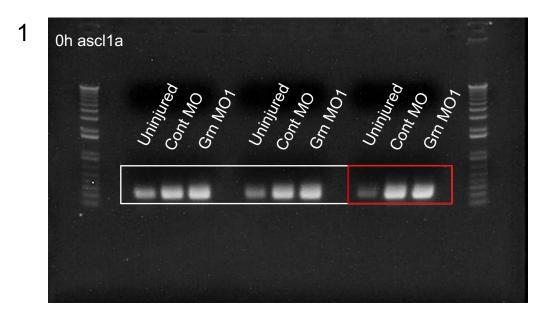


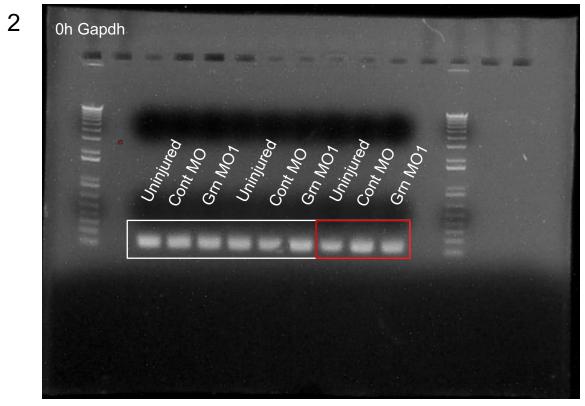


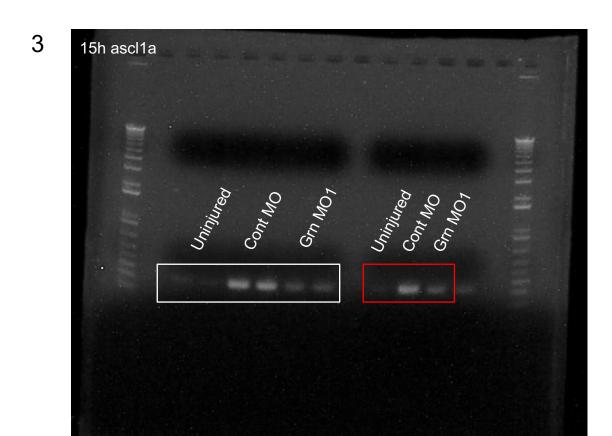


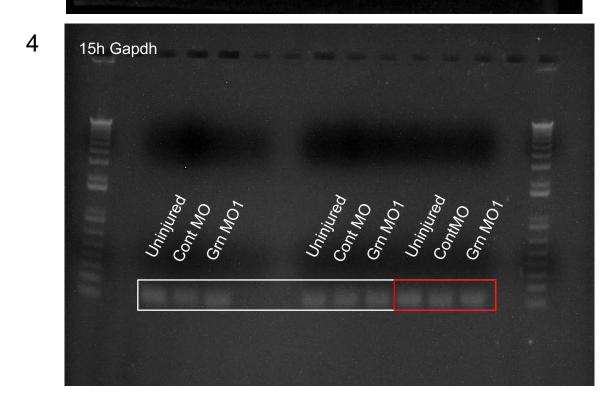


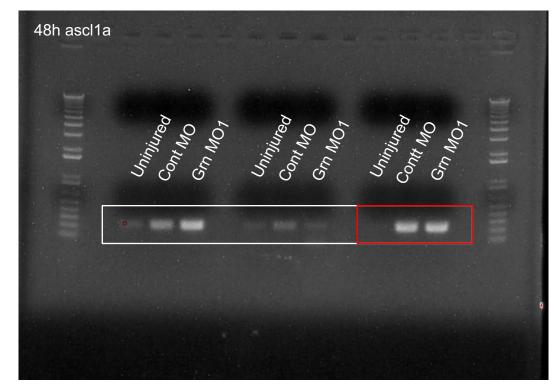




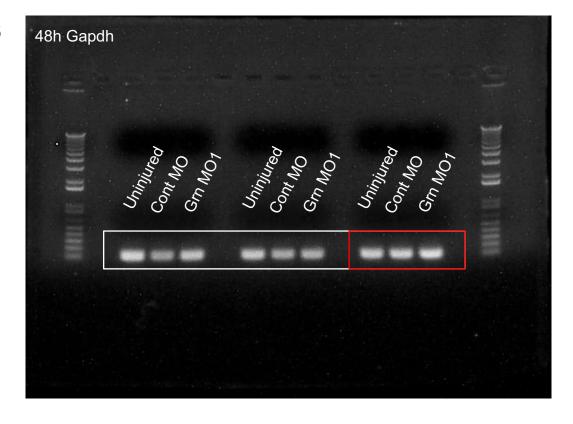










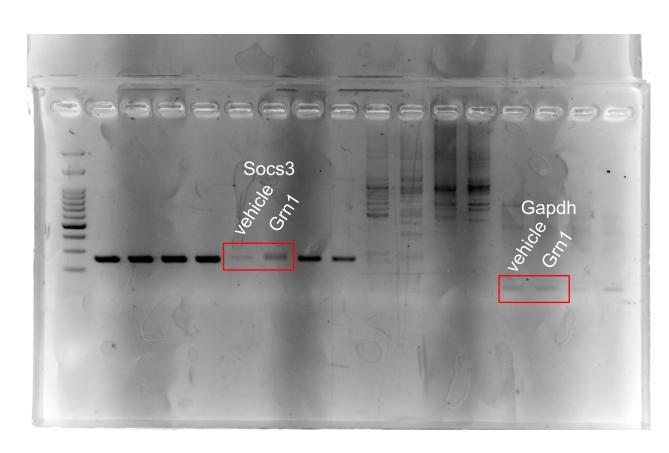


ascl1a

Lin28

Lin28

Lin28



Supplemental Figure legends

Figure S1. 4C4-negative, plastin-positive cells are observed at 24 hours post injury

Anti-4C4 and anti-plastin antibody staining at 24 hours post injury. White arrowheads indicate unmerged plastin positive cells which show neutrophils. Scale bar is $100 \ \mu m$.

Figure S2. Electroporation of MO into migrating cells

Lissamine-tagged control MO was electroporated at 15 h post injury. The eye was enucleated, fixed, and sectioned. The sections were stained by anti-4C4 antibody. Green shows 4C4-positive infiltrated cells. Red shows MO transduced cells. Merged image indicates MO was transduced into the infiltrated cells. Scale bar is 200 µm.

Figure S3. Representative images of BrdU positive cells by 15 h or 48 h EP groups

Green shows BrdU positive cells.

Figure S4. early expressed grn1 mRNA is not merged with plastin or mpeg.

Triple staining of *in situ* hybridization of *grn1*, plastin (neutrophil or microglia marker), and mpeg (macrophage or microglia marker) at 3 hours post injury (hpi). The merged area is punctured retinal tissue.

Figure S5 The full-length blots of results by RT-PCR

Representative blots showing the expression level of *ascl1a, grn1, grn2, grna, grnb* and *gapdh*. Red squares in the full-length blots are used for cropped blots in Figure 1.

Figure S6 The full-length blots of results by RT-PCR

Representative blots showing the expression level of *ascl1a* and *gapdh* at different times. White squares are full-length blots and red squares are used for cropped blots in Figure 3.

Figure S7 The full-length blots of results by RT-PCR

Representative blots showing the expression level of *ascl1a*, *lin28*, *socs3* and *gapdh*. White squares are full-length blots and red squares are used for cropped blots in Figure 4.