Supporting Information

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SI Text

Sequences Used for qRT-PCR. The forward (F) and reverse (R) primer sequences used for qRT-PCR are as follows: *ascl1a* (*achaete-scute complex-like 1a*): F 5′-CAACTGGTTTTGAGCGTTCG-3′, R 5′-GACATCCTCCCAAGCGAGTG-3′; *dlg7* (*discs, large homolog 7*): F 5′-AGGCGAGTCTCCTGTGGATG-3′, R 5′-TCCCTCTGTTCTGGGGTGAA-3′; *gpia*: F 5′-TCCAAGGAAACAAGCCAAGC-3′, R 5′-TTCCACATCACACCCTGCAC-3′; *hspd1* (*heat shock 60-kDa protein 1*): F 5′-AGGCTCTCTGGTGGTGGAGA-3′, R 5′-GCATCTAGCAGTGCCGTCCT-3′; *id3* (*inhibitor of DNA binding 3*): F 5′-TGCCATTAGGATGGATGAATGA-3′, R 5′-CGCAGATTGCTTTCCCACAC-3′; *mps1* (*monopolar spindle 1*): F 5′-ACTCGCAGGTCGGAACTCTG-3′, R 5′-CCACACGTCCCCTTTAGCAC-3′; *pcna* (*proliferating cell nuclear antigen*): F 5′-CATGATCTCGTGTGCCAAGG-3′, R 5′-TGAGCTGCACTGGCTCATTC-3′; *pdgfa* (*platelet-derived growth factor a*): F 5′-TTCCCCGAGAGCTGATTGAG-3′, R 5′-TGCTCCTTATGGTGGCCTTG-3′; and *six3b* (*sine oculis homeobox homolog 3b*): F 5′-CCAATCCGAGCAAGAAAAGG-3′, R 5′-CAGACTGCTTTGGCCCAGTC-3′.

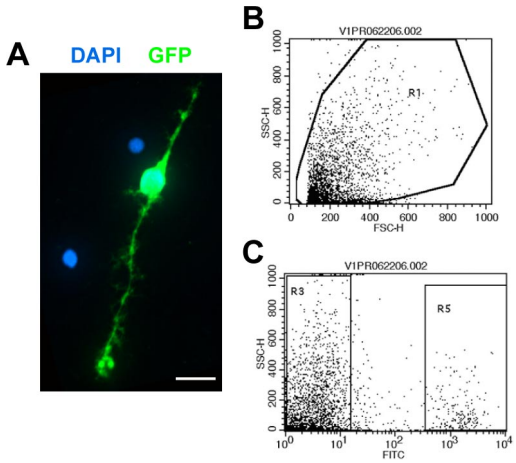

Fig. S1. Isolation of GFP$^+$ Müller glia. (*A*) Dissociated GFP$^+$ Müller glial cell (green). Counterstained with DAPI (blue). (*B* and *C*) Flow cytometry scatter plots. forward scatter-height (FSC-H); side scatter-height (SSC-H). Dissociated cells from adult *Tg(gfap:GFP)mi2002* zebrafish retinas were gated by forward and side scatters (*B*), and GFP$^+$ Müller glia were isolated based on fluorescence in the FITC channel (R5) (*C*). Our yield of dissociated retinal cells from adult zebrafish (5- to 6-month old) was $\sim 2.5 \times 10^5$ cells/retina, of which $\sim$9% were GFP$^+$ Müller glia. With flow cytometry, we could recover $\sim 2.1 \times 10^4$ Müller glia/retina, representing an efficiency of $\sim$84%. (Scale bar: 10 μm.)

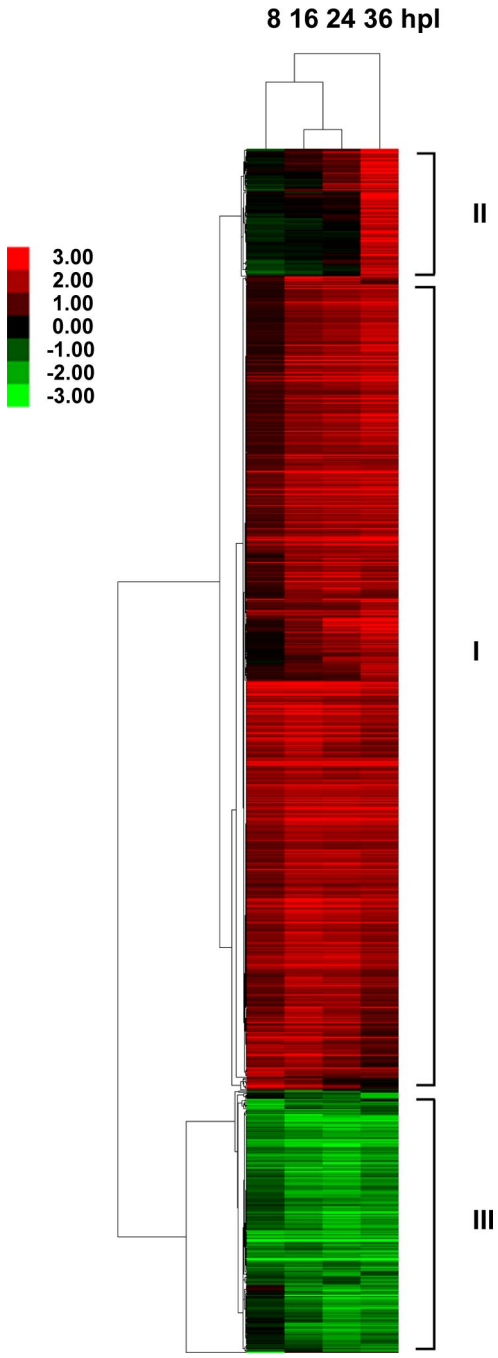

Fig. S2. Gene expression profiling of isolated Müller glia from intact and regenerating zebrafish retinas. ''Heat map'' fold changes of gene expression at 8, 16, 24, and 36 hpl relative to unlesioned retina on a $\log_2$ scale. Hierarchical clustering analysis revealed 3 major groups: I, II, and III.

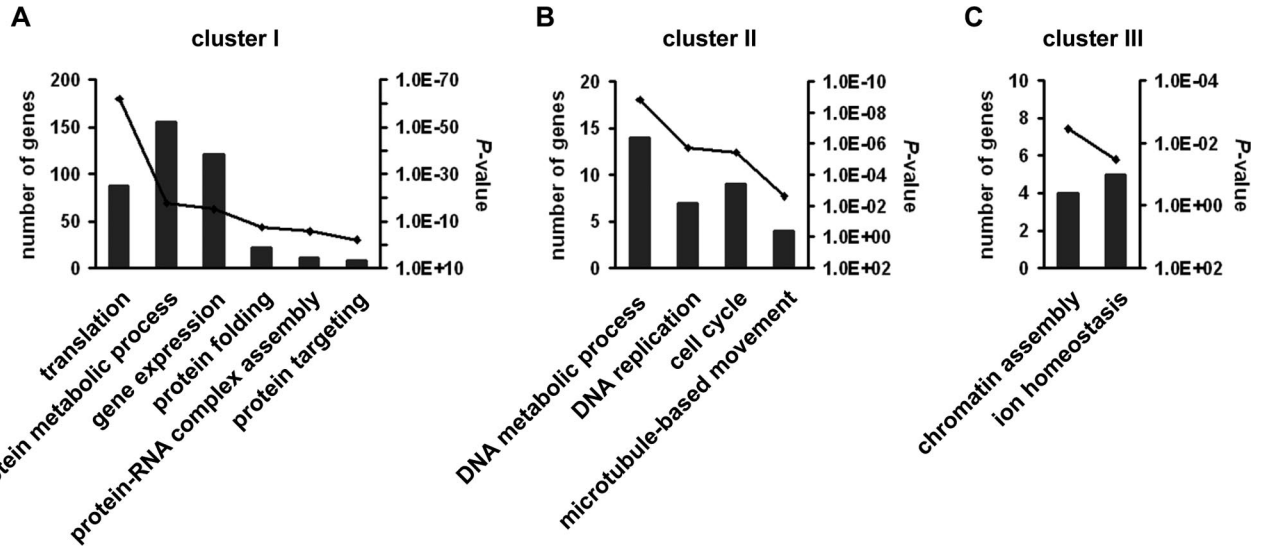

Fig. S3. Gene ontology grouping of genes within each cluster. Differentially expressed genes in the microarray analysis were subjected to hierarchical clustering, followed by functional and statistical analysis of the genes in each cluster. The number of genes in each biological process (columns) and the corresponding *P*-values (diamonds) are indicated. (*A*) Cluster I. (*B*) Cluster II. (*C*) Cluster III.

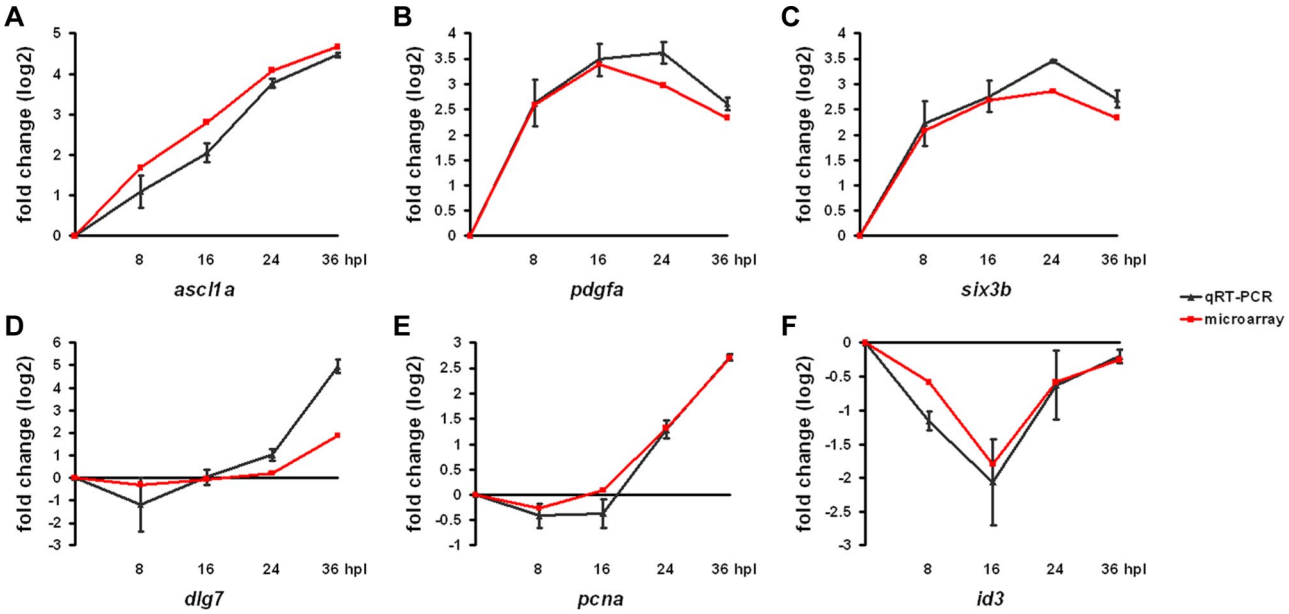

Fig. S4. qRT-PCR validation of expression patterns of selected genes. Expression fold changes of a subset of injury-responsive genes detected by qRT-PCR (gray) and microarray (red). (*A*–*C*) Genes from cluster I: *ascl1a*, *pdgfa*, and *six3b*. (*D* and *E*) Genes from cluster II: *dlg7* and *pcna*. (*F*) Gene from cluster III: *id3*. Error bars represent SEM for 3 independent biological replicates.

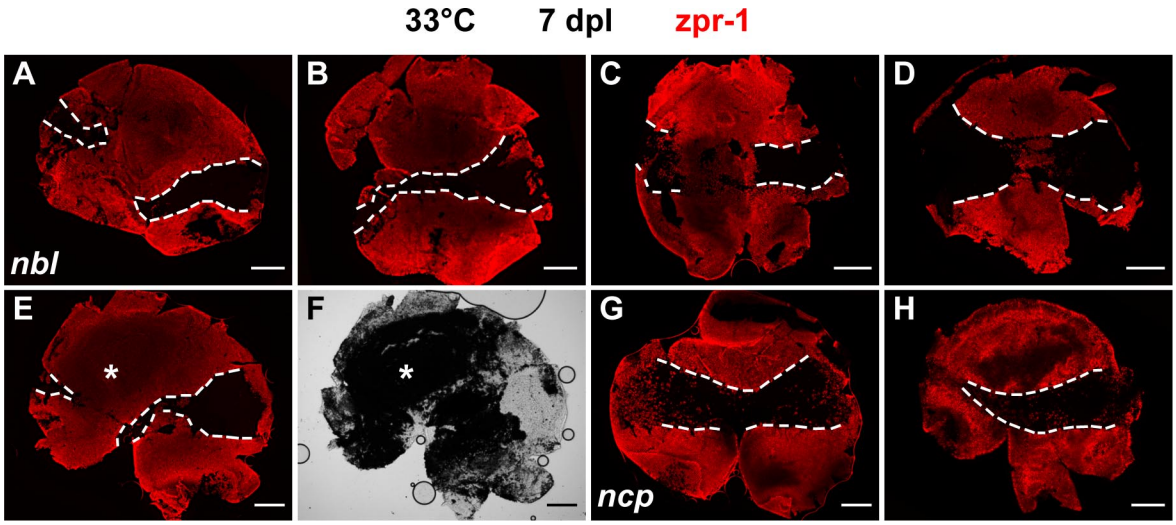

Fig. S5. Cone regeneration defect in *nbl* and *ncp* mutants at the restrictive temperature. (*A*–*E*, *G*, and *H*) Flat-mounted retinas at 7 dpl immunolabeled with zpr-1 (red). (*A*–*E*) One retina from each of 5 *nbl* mutants. (*F*) Bright-field image of *E*. (*G* and *H*) One retina from each of 2 *ncp* mutants. Dashed lines, light-damaged areas that have few or no zpr-1–labeled cones; we cannot determine from these preparations whether the rare scattered cones sometimes observed within the light-damaged areas survived the lesion or have regenerated. Asterisk, attached retinal pigment epithelium. (Scale bars: 300 μm.)

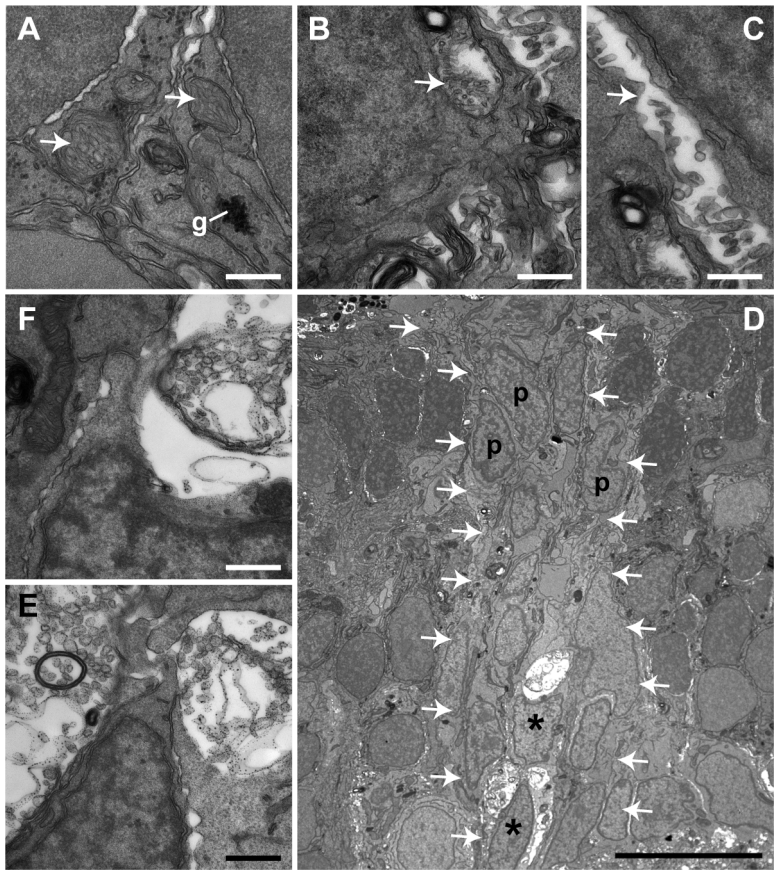

Fig. S6. Transmission electron micrographs of mitochondria in injury-activated Müller glia in WT siblings and *nbl* mutants after acute exposure to 33°C. (*A*–*C*) High-magnification images of mitochondria in injury-activated Müller glia in retinas at 2 dpl after 8 h of exposure to 33°C. See Fig. 4*D* for lower magnification images of these sections. (*A*) Glycogen granules (g) and mitochondria (arrows) in Müller glia in WT. (*B* and *C*) Swollen mitochondria with empty matrix in Müller glia of *nbl*. (*D*) Low-magnification view of a neurogenic cluster (within the arrows) in the inner nuclear layer of *nbl* at 3 dpl after 4 h of exposure to 33°C. Asterisks, Müller glia; p, progenitor. Note that the mitochondrial defect is present only in injury-activated Müller glia but not in the associated neuronal progenitors. (*E* and *F*) High-magnification images of mitochondria from the Müller glial cells in *D*. (Scale bars: 0.5 μm in *A*–*C*, *E*, and *F*; 10 μm in *D*.)

Table S1. Transcriptionally regulated genes common to regenerating retina, fin, and/or heart

Gene name	Gene symbol	Biological process
Monopolar spindle 1	*mps1* (***ttk***)	Cell cycle
Decorin	*dcn*	Cell signaling
Insulin-like growth factor binding protein 3	*igfbp3*	Cell signaling
Jagged 2	*jag2**	Cell signaling
Kallmann syndr. 1b	*kal1b*	Cell signaling
Meteorin	*metrnl*	Cell signaling
Platelet-derived growth factor α	*pdgfa*	Cell signaling
GLI-Kruppel family member GLI2a	*gli2a*	Cell signaling
TGF-β-induced	*tgfbi*	Cell signaling
TGF-β-induced factor homeobox 1	*tgif1*	Cell signaling
Activating transcr. factor 3	*atf3*	Immunoregulation
Clusterin	*clu*	Immunoregulation
LIM domain only 4	*lmo4*	Immunoregulation
Matrix metalloproteinase 14 beta	*mmp14b*	Immunoregulation
Similar to complement protein C7-1	*LOC570832**	Immunoregulation
Matrix metalloproteinase 9	*mmp9*	Immunoregulation
Suppressor of cytokine signaling 3b	*socs3b*	Immunoregulation
Tissue inhibitor of metalloproteinase 2	*timp2*	Immunoregulation
Cathepsin C	*ctsc*	Immunoregulation
Cathepsin B, a	*ctsba*	Proteolysis
Karyopherin alpha 2	*kpna2*	Protein import into nucleus
SRY-box-containing gene 11b	*sox11b*	Regulation of transcription
SRY-box-containing gene 4a	*sox4a*	Regulation of transcription
zic family member 2 (odd-paired-like) b	*zic2b*	Regulation of transcription
Nuclear receptor subfamily 1, group D, member 2b	*nr1d2b*	Regulation of transcription
Calreticulin, like 2	*calrl2*	Stress response
Heat shock 70-kDa protein 5	*hspa5*	Stress response
Heat shock 60-kDa protein 1	*hspd1*	Stress response

The genes listed are in the retinal microarray data set reported here and are also found in one or both of the 2 comparison data sets (1, 2). The boldfaced genes correspond to the temperature-sensitive regeneration mutants. All genes except *nr1d2b* are up-regulated at 1 or more sample times.
*Closely related gene is found in one of the comparison data sets: *jag1a* in fin; *C4–1* and *C4–2* in heart.

1. Schebesta M, Lien CL, Engel FB, Keating MT (2006) Transcriptional profiling of caudal fin regeneration in zebrafish. *Scientific World Journal* 6(Suppl 1):38–54.
2. Lien CL, Schebesta M, Makino S, Weber GJ, Keating MT (2006) Gene expression analysis of zebrafish heart regeneration. *PLoS Biol* 4:1386–1396.