

**Cell Biology:**

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Sheng Ding, Shaochen Chen and Kang Zhang  
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# Induction of Retinal Progenitors and Neurons from Mammalian Müller Glia under Defined Conditions\*

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Jack Jiagang Zhao<sup>†1</sup>, Hong Ouyang<sup>‡</sup>, Jing Luo<sup>†§§</sup>, Sherrina Patel<sup>‡</sup>, Yuanchao Xue<sup>§</sup>, John Quach<sup>‡</sup>, Nicole Sfeir<sup>‡</sup>, Meixia Zhang<sup>¶</sup>, Xiangdong Fu<sup>§</sup>, Sheng Ding<sup>||</sup>, Shaochen Chen<sup>\*\*</sup>, and Kang Zhang<sup>†##2</sup>

From the <sup>‡</sup>Department of Ophthalmology and Institute for Genomic Medicine, University of California, San Diego, La Jolla, California 92093, <sup>§</sup>Department of Cellular and Molecular Medicine and Institute for Genomic Medicine, University of California, San Diego, La Jolla, California 92093, the <sup>§§</sup>Department of Ophthalmology, The Second Xiangya Hospital, Central South University, Changsha, Hunan 41001, China, the <sup>¶</sup>Molecular Medicine Research Center, West China Hospital, Chengdu, Sichuan 610041, China, <sup>||</sup>Gladstone Institutes, University of California, San Francisco, San Francisco, California 94158-2261, the <sup>\*\*</sup>Department of NanoEngineering, University of California, San Diego, La Jolla, California 92093, and the <sup>##</sup>Veterans Administration Healthcare System, San Diego, California 92161

**Background:** Mammalian Müller glia are mitotic quiescent and committed.

**Results:** Loss of *p53* enhances Müller glia to proliferate and become progenitor-like cells, which differentiated to photoreceptors *in vitro* and incorporated into retina after transplantation.

**Conclusion:** Progenitor potential can be induced in mammalian Müller glia.

**Significance:** Induction of Müller glia stemness may serve as an exciting strategy for retinal repair and regeneration.

Vision impairment caused by loss of retinal neurons affects millions of people worldwide, and currently, there is no effective treatment. Müller glia of mammalian retina may represent an under-recognized and potential source for regeneration of a wide range of retinal cell types, including retinal ganglion cells and photoreceptors. Here, we demonstrated that mouse Müller glia cells have the capacity to be reprogrammed into the retinal neuronal cell fate and are competent to give rise to photoreceptors under a defined culture condition. Inactivation of *p53* released proliferation restriction of Müller glia and significantly enhanced the induction of retinal progenitor from Müller glia in culture. Moreover, following the ocular transplantation, the Müller glia-derived progenitors were differentiated toward the fates of photoreceptors and retinal ganglion cells. Together, these results demonstrate the feasibility of using Müller glia as a potential source for retinal repair and regeneration.

Retinal regeneration is a well known phenomenon across vertebrates. Müller glia has been demonstrated as a remarkable source of intrinsic regeneration for retinal progenitors in response to acute retinal injury in non-mammal vertebrates (1–3). In the acutely damaged retinas, Müller glial cells can dedifferentiate, reenter the cell cycle, and proliferate. These Müller glia-derived progenitors also express transcription factors found in embryonic retinal progenitors such as Pax6, Chx10, Six3, Ascl1a, Sox2, and

Sox9 (1). As demonstrated in chicken and zebrafish retinas, the Müller glia-derived progenitor cells produce new retinal neurons, which are able to integrate into the existing visual circuitry both structurally and functionally (1, 3, 4).

Although some evidence suggested that Müller glia in rodent and primate retinas may possess similar progenitor-like properties found in non-mammalian vertebrates (see review by Ref. 5), for some unknown inhibitory mechanism, the capability of spontaneous regeneration of retinal neurons by Müller glia is rather limited in mammals. It is of great interest to develop means that could eliminate the inhibitory barrier and enable mammalian Müller glia to regain progenitor characteristics. Among the possible molecular and cellular mechanisms in controlling neuronal regeneration, *p53*-mediated cell cycle arrest may play an important inhibitory role in preventing Müller glia proliferation in adult mouse retina (6). It has been reported that suppression of *p53* could significantly promote pluripotency and enhance the efficiency of induced pluripotent stem cell reprogramming by facilitating reentry into cell cycle of terminally differentiated somatic cells (7–12). We therefore investigated whether the inactivation of *p53* in Müller glial cells could potentiate the cells to regain progenitor-like characteristics and Müller glia serve as a potential source for regeneration of retinal neurons such as photoreceptors and ganglion cells under the permissible growth cues.

## EXPERIMENTAL PROCEDURES

*The Culture of Retinal Progenitor Cells from Müller Glia*—Eyeballs were enucleated from P6 mice, and the retinas were dissected free in DMEM. The retinas were digested with Papain dissociation system following the manufacturer's instructions (dissociation solution, DMEM/F12 containing 16.5 units/ml activated papain (Worthington, Lakewood, NJ) and 124 units/ml DNaseI (Sigma-Aldrich) at 37 °C for 15 min and maintained in stationary culture in 10% serum supplemented

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<sup>†</sup> To whom correspondence may be addressed. E-mail: j3zhao@ucsd.edu.

<sup>2</sup> To whom correspondence may be addressed. E-mail: kang.zhang@gmail.com.

## Müller Glia-derived Retinal Progenitors and Neurons

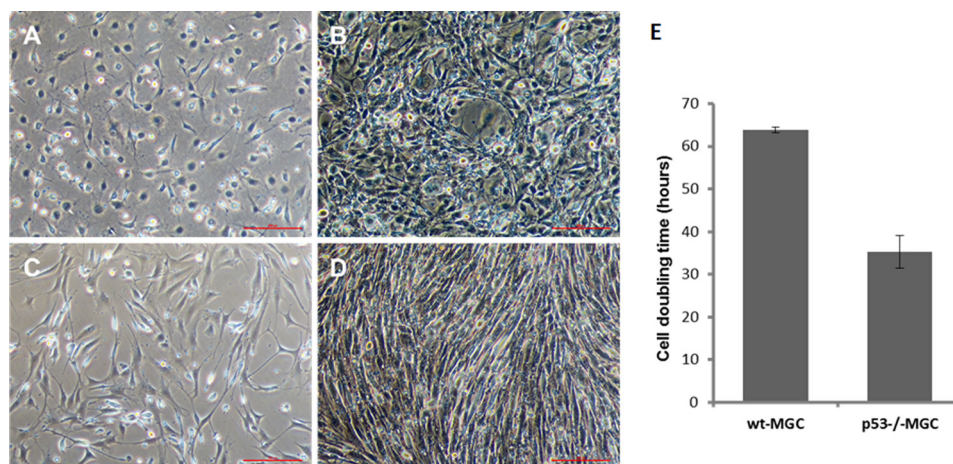


FIGURE 1. The phase contrast imaging of wt-MGCs (passage 1; **A**) and  $p53^{-/-}$  MGCs (passage 3; **B**) before induction and their corresponding progenitor-like cells after induction for 6 days (**C** and **D**) are shown. Spindle morphology was observed in  $p53^{-/-}$  cells (**D**), in comparison with the cells maintained in regular Müller glia culture condition (**B**). **E**, under the progenitor induction condition, the cell doubling time was ~64 and 35 h, respectively, for wild type and  $p53^{-/-}$  Müller glia cells. Scale bars, 100  $\mu\text{m}$ .

DMEM and F12 (1:1). The removal of aggregates and cellular debris after 6–7 days yielded purified Müller glial cell cultures, which could be maintained or passaged in DMEM/F12 with 20 ng/ml FGF, 20 ng/ml EGF, N2, and 0.2% BSA. For the induction of retinal progenitors from cultured Müller glial cells, the cells were grown in retinal progenitor induction medium, which is composed of DMEM/F12, 5% knock-out serum replacement, N2, 5 mM glutamine, 2 mM nicotinamide, 11.25  $\mu\text{g/ml}$  linoleic acid, 1  $\mu\text{M}$  Y27632, and supplemented with 20 ng/ml basic fibroblast growth factor; 20 ng/ml EGF; 10 ng/ml leukemia inhibitory factor. The confluent monolayer culture was dissociated with StemPro Accutase (Invitrogen) and can be passaged onto Matrigel-coated (BD Biosciences) plates.

**The Photoreceptor Differentiation**—Müller glia-derived retinal progenitors (MRPs)<sup>3</sup> were cultured in photoreceptor differentiation medium (DMEM/F12:Neurobasal medium 1:1, N2, B27, 0.05% BSA fraction V; 2 mM Glutamax, 50 nM docosahexaenoic acid, and supplemented with 1  $\mu\text{M}$  IWP2, 10  $\mu\text{M}$  *N*-[(3,5-difluorophenyl)acetyl]-*L*-alanine-2-phenylglycine-1,1-dimethylethyl ester, 100 nM purmorphine, 100 nM retinoic acid, 100  $\mu\text{M}$  taurine, 10 ng/ml basic fibroblast growth factor) for 2 weeks.

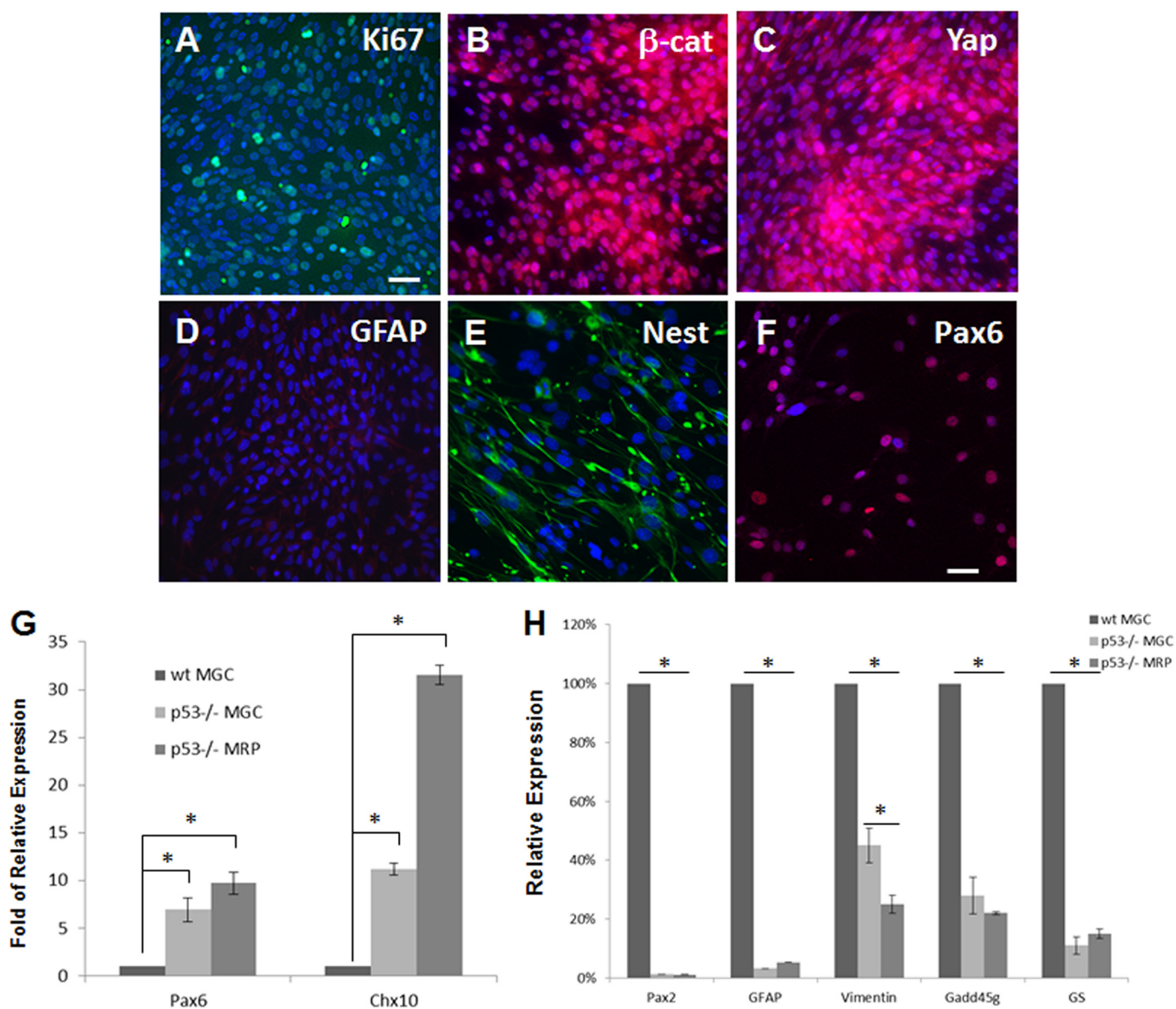
**RNA Isolation, Reverse Transcription, and Quantitative PCR Analysis**—Total cellular RNA was isolated from cells using the RNeasy kit (Qiagen), and cDNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad), both following the manufacturer's instructions. The transcripts were amplified for 40 cycles using Power SYBR Green PCR Master Mix on 7500 Real Time PCR System (Applied Biosystems). Measurements were performed in triplicates and normalized to GAPDH levels. Gene-specific primer sequences were used as follows: *Pax6*, 5'-CTGAGGAACCAGAGAAGACAGG-3' (forward) and 5'-CATGGAACCTGATGTGA AGGAGG-3' (reverse); *Chx10*, 5'-CCACCTTCTT GGAAGTGCTGTC-3' (forward) and 5'-GTCACCTGGAGGAAACATCTTCGG-3' (reverse); *Pax2*, 5'-CAGCCTTCCACCCAACG-3' (forward) and 5'-GTGGCG-

GTCATAGGCAGC-3' (reverse); *GFAP*, 5'-CACCTACAGG-AAATTGCTGGAG G-3' (forward) and 5'-CCACGATGTTCTCTTGAGGTG-3' (reverse); vimentin, 5'-CGGAAAGTGAATCCTTGCA-3' (forward) and 5'-CACATCGATCTGGACATGCTGT-3' (reverse); *Gadd45g*, 5'-GGGAAAGCACTGCACGAACT-3' (forward) and 5'-AGCACGCAAAGGTCACATTG-3' (reverse); glutamine synthetase 5'-CTGATGGATGGCCCTACTGT-3' (forward) and 5'-CAACTTCCCGGTACTGCAT-3' (reverse); *Crx*, 5'-TATGCACGTGAGGAGGTTGCT-3' (forward) and 5'-CTGTCTGC ATTTCCGCCCTACG-3' (reverse); *Nr2e3*, 5'-GCCTTATCACCGCCGAAACTTG-3' (forward) and 5'-CATGGATGCCATCCAGACTGCA-3' (reverse); *Pde6b*, 5'-TGGAGAACCGTAAGGACATCGC-3' (forward) and 5'-TCCTCACAGTCAGCAGGCTCTT-3' (reverse); *Gnat1*, 5'-GCTTGTGGAAGGACTCGGTAT-3' (forward) and 5'-AACGCAACACGTCCTGCTCAGT-3' (reverse); arrestin, 5'-CGAAGACTGTGTTTGTTCAGGA-3' (forward) and 5'-AGGCCCTGCTTCTGACAGT-3' (reverse); GAPDH, 5'-GTCAAGGCCGAGAATGGGAA-3' (forward) and 5'-TTGGCTCCACCCTTCAAGTG-3' (reverse).

**Immunofluorescence Staining**—For immunocytochemistry, cells were fixed in 4% paraformaldehyde for 20 min at room temperature, permeabilized with 0.3% Triton X-100 in PBS for 5 min twice, and blocked with 5% normal donkey serum in PBST (0.3% Triton X-100), followed by an overnight incubation in primary antibody solutions at 4 °C. After three washes in PBS, cells were incubated with Alexa Fluor fluorescently conjugated secondary antibodies for another 90 min. After rinses and washes in PBS, cell nuclei were counterstained with Hoechst 33342 (100 ng/ml) for 10 min. Primary antibodies and their working dilutions were as follows: rabbit anti-Pax6 (1:600, Covance), mouse anti-Ki65 (1:100, BD Biosciences), rabbit anti- $\beta$ -catenin (1:100, Cell Signaling), rabbit anti-GFAP (1:1000, Dako), mouse anti-nestin (1:1000, BD Biosciences), rabbit anti-recoverin (1:2000, Millipore), mouse anti-rhodopsin (1:250, Millipore), rabbit anti-red/green opsin (1:300, Millipore), rabbit anti-Tuj1 (1:2000, Covance), rabbit anti-Yap (1:200, Santa Cruz Biotechnology), and mouse anti-RFP (1:500, Pierce Biotech-

<sup>3</sup> The abbreviations used are: MRP, Müller glia-derived retinal progenitor; IRBP, interphotoreceptor retinoid binding protein; RFP, red fluorescent protein.





**FIGURE 2. Induction of retinal progenitor characteristics from  $p53^{-/-}$  MGCs.** An increased number of cells entering cell cycle was observed as evidenced by Ki67 expression (A). In addition, nuclear translocation of  $\beta$ -catenin ( $\beta$ -cat; B) and Yap (C) were observed. Markers of retinal progenitor such as nestin (Nest; E) and Pax6 (F) were becoming positive and the MGC marker GFAP (D) was greatly diminished after induction. Scale bars, 30  $\mu$ m (A–F). Shown is a quantitative PCR analysis of the expression of selected markers in  $p53^{-/-}$  MRP (G and H). Fold increase in the expression level of retinal progenitor transcription factors Pax6 and Chx10 was observed after induction (G). In contrast, the expression of Müller glial cell markers such as Pax2, GFAP, vimentin, Gadd45g, and glutamine synthetase (GS) were significantly reduced (H). The error bars represent S.E. \*,  $p \leq 0.05$ .

nology), rabbit anti-GFP (1:1000, Invitrogen), or chicken anti-GFP (1:400, Invitrogen). The secondary antibodies used were the corresponding Alexa Fluor 488, 555, 633, or 647 fluorescently labeled antibodies (1:1000, Invitrogen). Labeled cells were imaged with a laser-scanning confocal microscope (Olympus FV1000). The specific immunoreactivity of each antibody was confirmed by immunostaining with appropriate retinal tissues as a positive control under the same conditions.

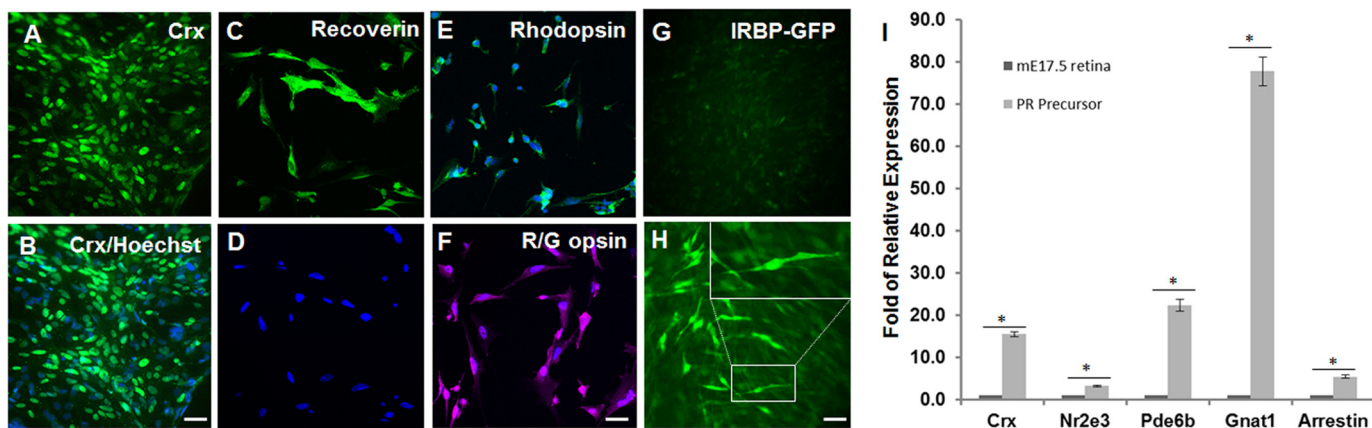
**Cell Transplantation**—We performed transplantation experiments following published protocols with some minor modifications (13, 14). Briefly, RFP-labeled MRP derivatives were dissociated into single cells with StemPro Accutase (Invitrogen) and concentrated to a density of  $5 \times 10^4/\mu$ l in balanced salt solution (Alcon). With a glass micropipette, 1–2  $\mu$ l of cell sus-

pension was slowly injected into the vitreous and subretinal spaces of eyes of 15-day-old NSG mice (The Jackson Laboratory, Bar Harbor, ME). At different time points post-transplantation, the animals were sacrificed, and the eyes were enucleated and embedded in tissue freezing medium, cryosliced, and co-immunostained as described above. Animal procedures were conducted with the approval and under the supervision of the Institutional Animal Care and Use Committee at the University of California, San Diego.

## RESULTS

**Dedifferentiation of Müller Glia to Retinal Progenitor-like Cells**—As demonstrated recently, loss of  $p53$  could enhance the proliferation of Müller glia (6) and enhance induced pluripo-

## Müller Glia-derived Retinal Progenitors and Neurons



**FIGURE 3. Photoreceptor precursors are induced from  $p53^{-/-}$  MRP.** More than 80% of cells expressed Crx, a photoreceptor progenitor specific marker, after induction for 5 days (A and B, green), cell nuclei were counter stained with Hoechst 33342 (B, blue). After further culturing in photoreceptor differentiation medium for ~10 days, photoreceptor-specific markers such as recoverin (C) with nuclei counterstaining (D), rhodopsin (E) and red/green opsin (R/G opsin; F) were detected by immunostaining. IRBP-GFP reporter expression was barely visible before the photoreceptor differentiation of  $p53^{-/-}$  MRPs (G) but was brightly visible and showed photoreceptor-like morphology after differentiation for 10 days (H). Scale bars, 25  $\mu$ m (A–H). I, quantitative PCR analysis of gene expression in photoreceptor precursors derived from  $p53^{-/-}$  MRP after 2 weeks of differentiation in culture. Induced expression of photoreceptor markers such as precursor transcription factors Crx and Nr2e3, cone cell-specific marker Arrestin, and rod-specific markers Pde6b and Gnat1 was detected. The error bars represent S.E. \*,  $p \leq 0.05$ .

tent stem cell reprogramming (8). To test whether  $p53$  null Müller glia could be readily converted into progenitor-like cells in culture, we isolated the Müller glial cells from the neural retina of  $p53$  knock-out mice. In agreement with previous report (6), we found that  $p53^{-/-}$  Müller glia maintains its proliferative state through the culture *in vitro* and can be propagated for generations (Fig. 1, A and B). Therefore, we used  $p53^{-/-}$  Müller glia to ask whether once reentering cell cycle, mammalian Müller glia is more likely to gain progenitor-like characteristics and can be further directed toward a specific neuronal fate under a defined condition. To test this hypothesis, neural retinas of postnatal day 6 eyes of  $p53^{-/-}$  mice were dissected and dissociated to single cell suspension, followed by seeding on a Matrigel-coated plate. After a week culturing in regular Müller glia growth medium, the cells proliferated and formed a confluent monolayer. The proliferating  $p53^{-/-}$  Müller glia could be continuously passaged *in vitro* for at least 10 passages. Following the establishment of primary Müller glia culture, the cells were subjected to dedifferentiation in a serum-free and chemical defined medium. After three to four days, the cells rapidly formed a confluent monolayer and displayed a spindle-like morphology (Fig. 1D). The calculated doubling time for the  $p53^{-/-}$  MRPs is ~35 h, which is similar to the doubling time 31~36 h reported previously for  $p53^{-/-}$  Müller glia culture (6). The  $p53^{-/-}$  MRP continued to be proliferative as indicated by numerous Ki67-positive cells in the culture (Fig. 2A). To explore the plausible involvement of some key stem cell/progenitor signaling pathways, such as *Wnt* and *Hippo* signaling pathways (15–17) in the conversion of Müller glia to retinal progenitor, we performed immunocytochemistry to examine the subcellular localization of key players in these two pathways. Interestingly, we found that  $\beta$ -catenin, a major player of *Wnt* pathway, and Yap, a major downstream effector of *Hippo* signaling pathway, were both accumulated in nucleus (Fig. 2, B and C) even at high cell density. The staining result suggested that these two well documented progenitor-promoting factors may be actively involved in induction of retinal pro-

genitor-like cells from Müller glia. In addition, the elevated expression of retinal progenitor markers such as nestin, Pax6, and Chx10 was detected after the induction (see Fig. 2, E–G, respectively). Although the loss of  $p53$  usually elevates *GFAP* expression in Müller glia (6), under our serum-free and defined condition for progenitor induction,  $p53^{-/-}$  MRPs showed the significant down-regulation of *GFAP* (Fig. 2, D and H). The expression of a typical glial transcription factor *Pax2* and Müller glial cell markers such as Gadd45g, glutamine synthetase, and vimentin were also decreased significantly (Fig. 2H).

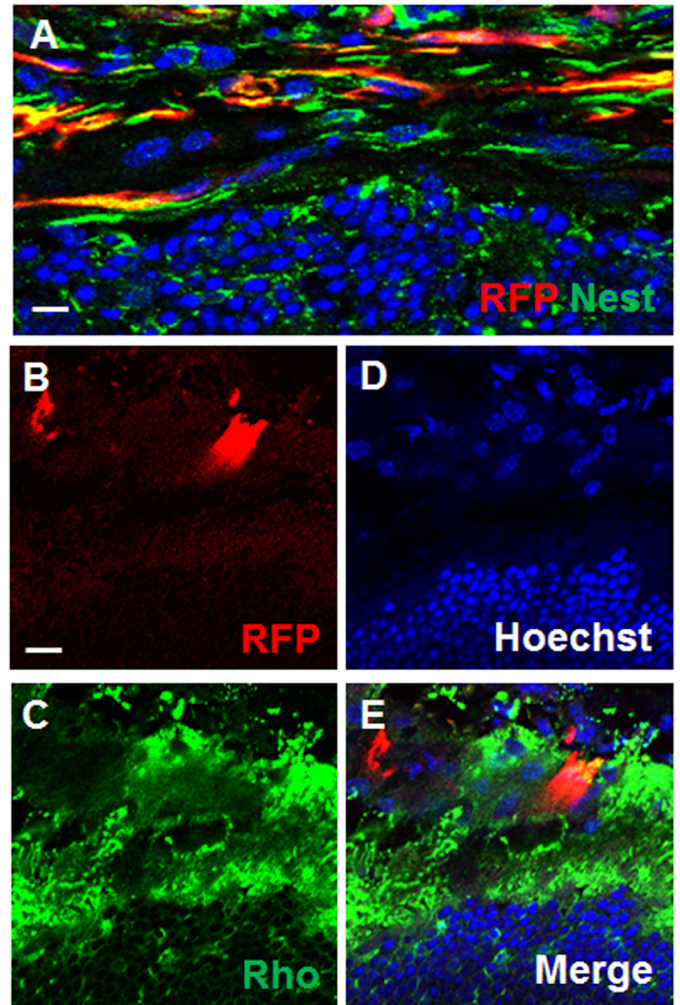
**Induction of Photoreceptor Precursors from  $p53^{-/-}$  MRPs—**To determine whether Müller glia-derived retinal progenitor-like cells have the potential to differentiate toward photoreceptor fate *in vitro* in a similar fashion as seen in cases of photoreceptor induction from primary retinal progenitors and embryonic stem cell-derived retinal progenitors (18, 19), we cultured  $p53^{-/-}$  MRPs in a defined medium that is favorable for photoreceptor induction. Differentiation was initiated by withdrawal of EGF and LIF. Previous studies demonstrated that inhibition of *Notch* and *Wnt* signaling is important for retinal progenitor differentiation. In addition, sonic hedgehog (shh) and retinoic acid were used along with *N*-[(3,5-difluorophenyl)acetyl]-L-alanyl-2-phenylglycine-1,1-dimethylethyl ester, a  $\gamma$ -secretase inhibitor that could significantly increase the formation of photoreceptor precursors by suppressing *Notch* signaling, to direct the photoreceptor lineage commitment of retinal progenitors in culture (20, 21). Therefore, we included small molecular inhibitors IWP2 and *N*-[(3,5-difluorophenyl)acetyl]-L-alanyl-2-phenylglycine-1,1-dimethylethyl ester for suppressing *Wnt* and *Notch* signaling during the photoreceptor induction. Moreover, based on the previous reports (19, 22), we used purmorphamine, a small molecular agonist of *Hedgehog* pathway, retinoic acid, and taurine to promote neuronal fate restriction toward photoreceptor in culture. After 5 days in culture, >80% cells expressed Crx, a key transcription factor for initiating photoreceptor lineage differentiation (Fig. 3, A and B), indicating an massive induction of photoreceptor precu-



sors from MRPs can be achieved efficiently in a monolayer culture system.

**Differentiation of Photoreceptors from Photoreceptor Precursors**—Next, we tested whether the MRP-derived Crx<sup>+</sup> photoreceptor precursors could be further differentiated to more mature photoreceptors containing visual pigments. The photoreceptor precursors were continuously cultured in the photoreceptor differentiation medium. After 10 more days, ~20% cells started to exhibit characteristic neuronal morphology and showed positive staining for various photoreceptor markers, including recoverin of immature photoreceptors (Fig. 3C), red/green opsin (Fig. 3F) of cone photoreceptors, and rhodopsin (Fig. 3E) of rod photoreceptors. To determine whether photoreceptors differentiated from *p53*<sup>-/-</sup> MRPs *in vitro* also express interphotoreceptor retinoid binding protein (IRBP), a marker of both rod and cone photoreceptors (23), we infected MRPs with an IRBP-GFP lentivirus and differentiated the transduced cells toward the photoreceptor fate. The IRBP-GFP labeling approach has been used to specifically mark photoreceptors in transgenic mice and in human, mouse, and chick retinal explants (24, 25). After 2 weeks of differentiation, >90% cells became GFP-positive. By day 20, clusters of bright IRBP-GFP-expressing photoreceptors (>20% of population) displayed typical morphological features such as a short inner process and long extended outer process, similar to an outer segment (Fig. 3H). Moreover, quantitative PCR analysis showed significant up-regulation of photoreceptor specific markers after the 15 days of induction. In comparison with mouse neural retina at embryonic day 17.5, the cultured photoreceptors showed ~78- and 22-fold increase of expression of rod specific proteins Gnat1 and Pde6b, respectively, and ~5.4-fold increase of cone arrestin expression (Fig. 3I). We noticed that the photoreceptor differentiation showed the preference toward the fate of rod, as evidenced by the relatively high expression level of rod specific markers Gnat1 and Pde6b (Fig. 3I) but relatively small increase of cone marker arrestin.

**Retinal Transplantation of *p53*<sup>-/-</sup> MRP Derivatives**—Next, we investigated whether MRP-derived differentiated cells are able to survive and differentiate into different types of retinal neurons after being injected into the rodent eye. To track the fate of transplanted cells *in vivo*, the cells were transduced with a RFP-expressing lentivirus. To avoid immune rejection of transplanted cells, the NSG immunodeficiency SCID mice were used for cell grafting. ~5 × 10<sup>4</sup> cells in 1–2 μl volume were injected into the subretinal as well as epi-retinal spaces of 2-week-old animals. Overall, six eyes were injected and enucleated at 3 weeks post-transplantation. In the subretinal region, majority of grafted RFP<sup>+</sup> cells were found near the injection site. Although many of these did not migrate into the photoreceptor layers, they exhibited neuronal characteristics such as elongated cell processes and continuously expressed nestin, an immature neuronal marker (Fig. 4A). A close examination of retinal sections of grafted eye, we observed that some RFP<sup>+</sup> cells moved into the outer segment layers were also positive for rhodopsin immunostaining (Fig. 4, B–E). The data indicates that MRP-derived precursors may survive and commit to the photoreceptor fate in the grafted tissue and are capable of



**FIGURE 4. Transretinal transplantation of *p53*<sup>-/-</sup> MRPs in immunodeficient mice.** A, RFP-tagged grafted cells (red) were detected in the subretinal space 3 weeks post transplantation, and a majority of these were still positively stained for nestin (RFP Nest; green), a marker for immature progenitors, and localized next to the photoreceptor layer. B–E, a subset of incorporated cells expressed rhodopsin (green), a rod photoreceptor-specific marker. Scale bars, 25 μm (A) and 20 μm (B–E).

expressing rod-specific proteins. Moreover, we observed the transplanted cells attached onto the retina to form an epi-retinal tissue (Fig. 5). Some of these RFP-tagged cells were co-staining for Islet1 and Brn3, two transcription factors known for their role in driving retinal ganglion cell formation (Fig. 5, A–D). In addition, migration of transplanted cells into the RGC layer was observed (Fig. 5, H–J). These results indicate that *p53*<sup>-/-</sup> MRP has the ability to commit to different retinal cell fates *in vivo*.

## DISCUSSION

Unlocking mammalian Müller glia regenerative potential to replace lost retinal cells has long been a fascinating topic. In this report, we investigated whether mouse Müller glia could be redirected to the different fates of retinal neurons and serve as a potential source for retinal regeneration. We found that Müller glia can be reversely dedifferentiated to retinal progenitors under a serum-free, chemically defined condition. It has been well known that proliferation of mammalian Müller glia is slow

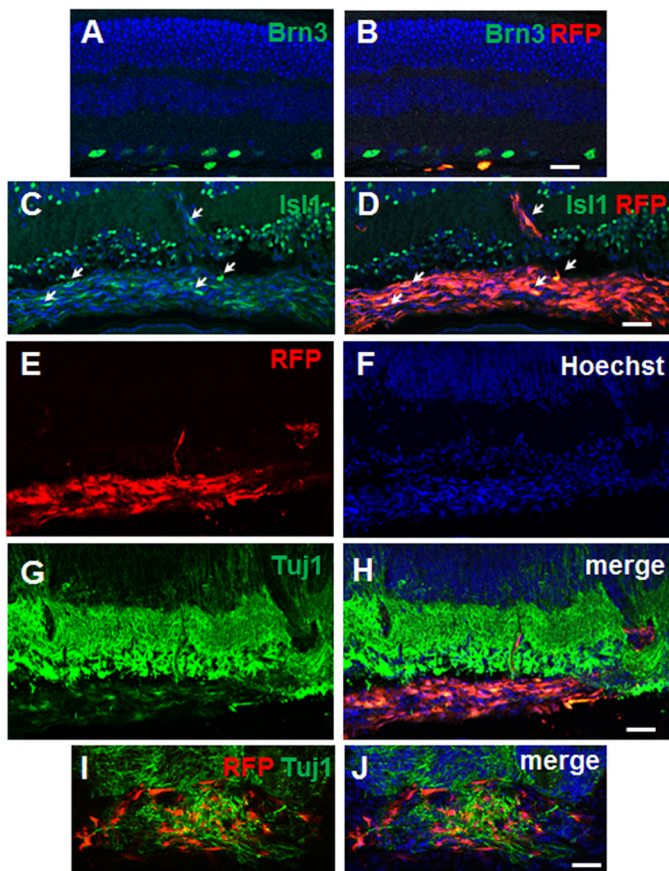


FIGURE 5. The transplanted RFP-tagged  $p53^{-/-}$  MRPs (red) formed an epi-retinal tissue onto the retinal ganglion cell layer 3 weeks post transplantation. A subset of cells expressed retinal ganglion cell markers such as Brn3 (A and B), Islet1 (C and D, small white arrows) and Tuj1 (E and J). Incorporation of transplanted cells into the retinal ganglion cell layer was also observed (H–J). Scale bars, 25  $\mu\text{m}$  (A and B) and 50  $\mu\text{m}$  (C–J).

and inefficient, making it difficult to generate Müller glia cell lines for characterization and transplantation. We showed that loss of tumor suppressor  $p53$  activity could significantly enhance the proliferation potential of cell and facilitate the reprogramming process of Müller glia into retinal progenitor cells. Our finding is corroborated by a recent report that down-regulation of  $p53$  is required for cell cycle reentry of postmitotic differentiated cells during the blastema formation stage of *Salamander* limb regeneration (26). We also showed potential involvement of *Wnt* and *Hippo* signaling pathways in de-differentiation and acquiring stemness as evidenced by nuclear accumulation of  $\beta$ -catenin and Yap protein in  $p53^{-/-}$  Müller glia-derived retinal progenitors. In addition, we demonstrated that photoreceptor precursors can be rapidly and efficiently induced from  $p53^{-/-}$  Müller glia-derived retinal progenitors *in vitro*. Furthermore, the transplanted  $p53^{-/-}$  Müller glia-derived progenitors were able to incorporate into the host retina and expressed either retinal ganglion cell or photoreceptor markers, such as Islet1 and Brn3 or rhodopsin and IRBP, respectively. Importantly, we did not observe tumor formation in any of these transplanted animals, despite lacking of tumor suppressor  $p53$  activity in the donor cells. This result is in alignment with the observation there are no documented cases of tumors of Müller glial cell origin.

Müller glia cell is the last differentiated cell type during retinal neurogenesis after differentiation of all of retinal neurons during mammalian retinal development (27). A similar scenario exists in the adult mammalian brain in which glial cells, the equivalent of Müller glia, is the last differentiated cell type during neurogenesis after differentiation of all cortical neurons. It has been found that radial glial-like cells of hippocampus behave similar to resident, dormant adult neural stem cells and can be activated upon stimulation or injury (28).

Using pluripotent stem cells to regenerate and repair damaged tissues holds great promise; however, it also faces significant safety hurdles such as tumor formations and genomic mutations (29). Endogenous adult stem cells provide an alternative source of regeneration, as they will not have issues of tumor formation or transplant rejection. In this regard, glial cells could be an ideal source of neural repair and regeneration.

Our results provide a new perspective on the role of  $p53$  in restricting mammalian Müller glia potential for retinal neuronal regeneration, and form a basis toward *in vivo* repair and regeneration of photoreceptors as well as retinal ganglion cells by Müller glia cell-based therapy.

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