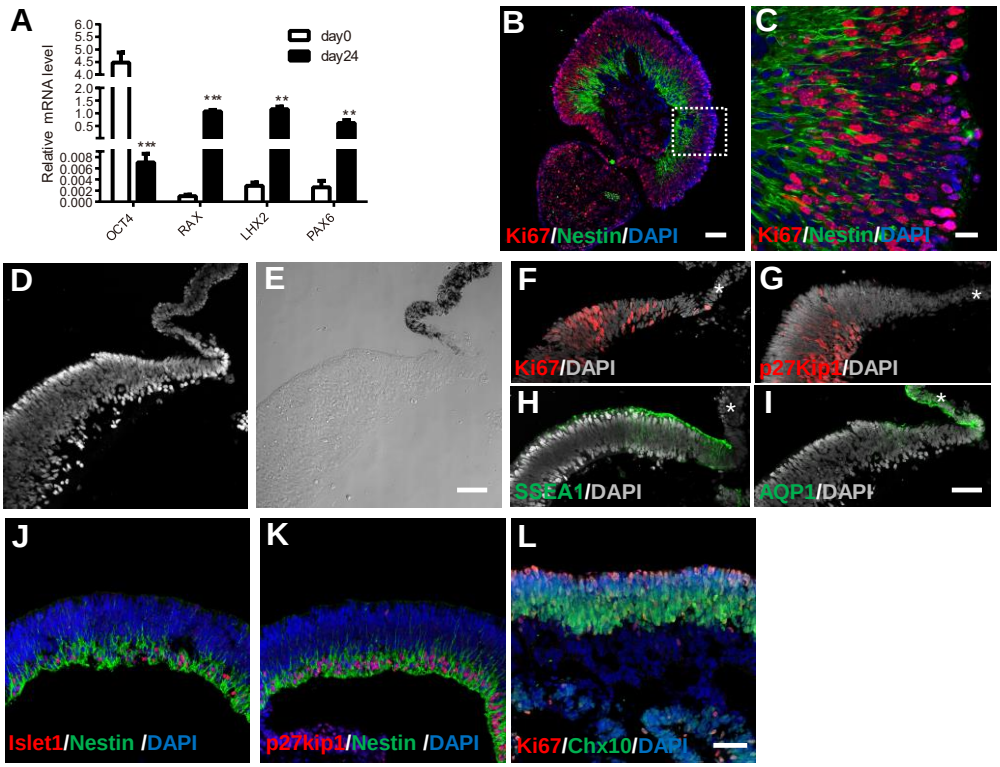

Figure S1. Identification and characteristics of the retinal organoid and CMZ. (A) RT-PCR analysis of mRNA of OCT4, Rax, LHX, and Pax6 in the 3D retinal organoid at D0 and D24. The pluripotent marker OCT4 was robustly downregulated after retinal differentiation while the eye field transcription factors Rax, LHX2, and Pax6 were dramatically upregulated after the formation of the 3D OC. (Mean±SD; D0, n=9; D24, n=9, 3 independent experiments) **p < 0.01; ***p < 0.005. **(B,C)** Immunostaining of 3D human retinal organoids at D24 for Nestin and Ki67. **(D,E)** DAPI staining and a differential interference contrast (DIC) image of the CMZ showing the specific cell shape and arrangement in the CMZ. **(F~I)** Immunostaining of the CMZ at D30 for Ki67 (F), P27kip1 (G), SSEA1 (H), and AQP1 (I). **(J)** Immunostaining of Islet1 and Nestin shows the Islet1^{+} ganglion progenitor cells were colocalized with Nestin at D24. **(K)** Nestin is expressed in the cell body of inner cell layer at D24 in which the CDK inhibitor p27Kip1 is present. **(L)** Ki67+ was distubuted at the most apical layer of the uncommitted Chx10+ retinal progenitor cells. Scale bars: 20 µm (C); 50 µm (D~I), 100 µm (B, J~L).

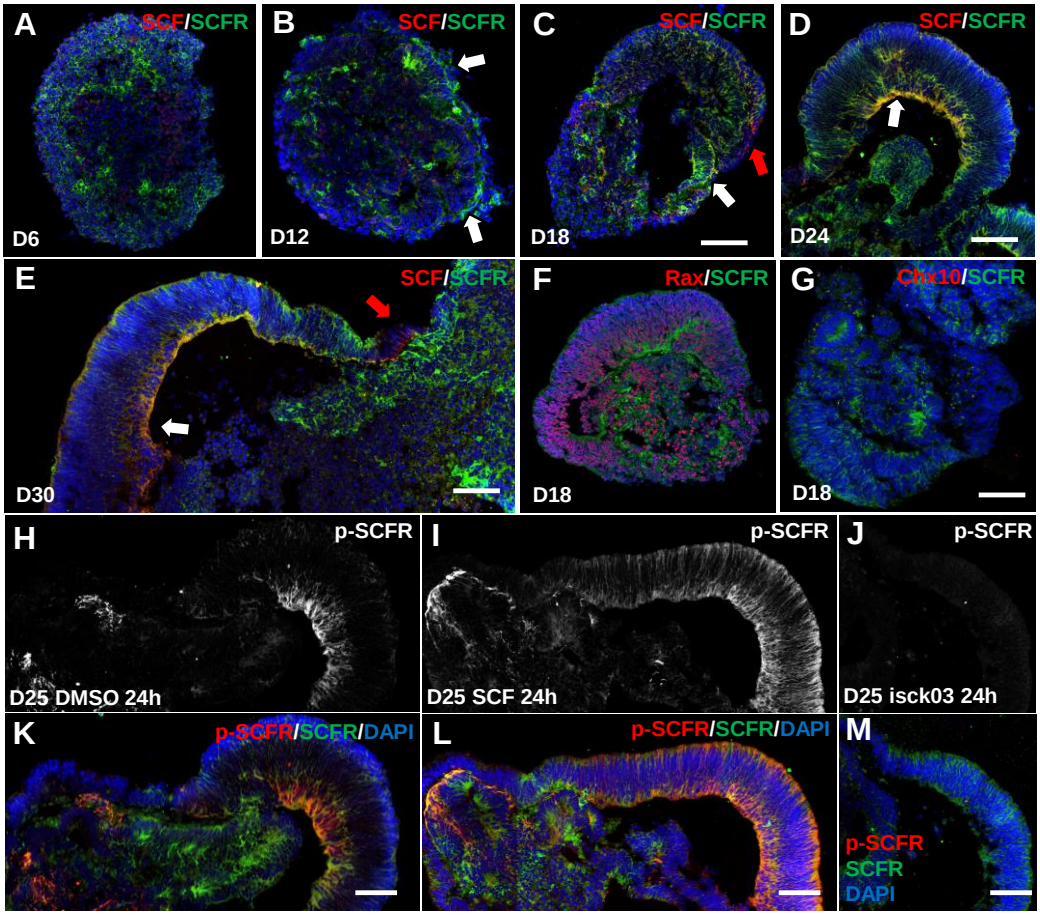

Figure S2. Spatiotemporal expression pattern of SCF and SCFR during the development of retinal organoid and activation of SCF/SCFR signaling pathway.

(A~E) SCFR first emerged at the outer portion of embryonic body at D6(A), gradually enriched at the epithelium-like tissue and cells at D12(B, white arrow), robustly located at the optic vesicle and neural retina at D18 and D24(C, D, white arrow), and preferentially maintained highly expressed in the CMZ and negatively expressed in the RPE at D30(E, white arrow). SCF was not expressed until D18 when the original optic vesicle formed(A~C, red arrow). Location of SCF mainly included two domain: basal side of the central SCFR positive NR and SCFR negative tissue(RPE), meanwhile hardly expressed in the CMZ(D, E, red arrow). **(F~G)** The Rax^{+} optic vesicle was first emerged at D18 when the neural retina marker Chx10 was not expressed yet. **(H~M)** p-SCFR signal robustly emerged in the basal side of the central NR at D25(H, K); p-SCFR was enhanced by SCF(I, L)and inhibit by isck03(J, M) Scale bars: 100 μm (A~D, F, G); 200 μm (E, H~M).

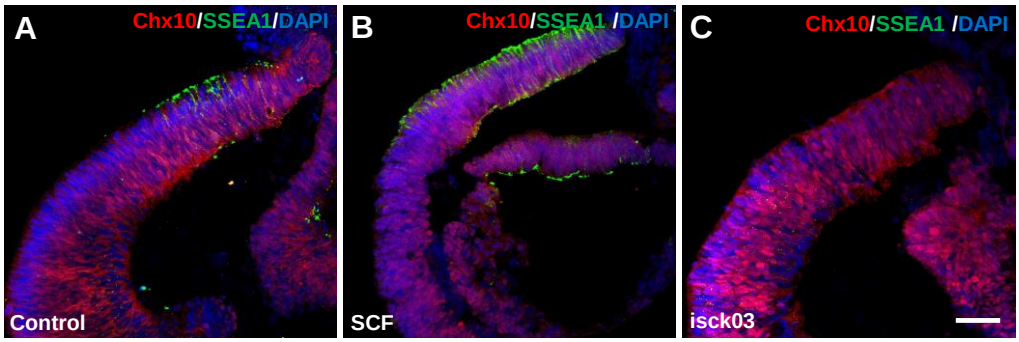

Figure S3. The border of CMZ and NR delimitated by the staining of SSEA1 and Chx10. (A~C) Double-staining of SSEA1 and Chx10 in the control (A), SCF (B), and isck03 (C) treatment group at D30. Scale bars: 100 μm (A~C)

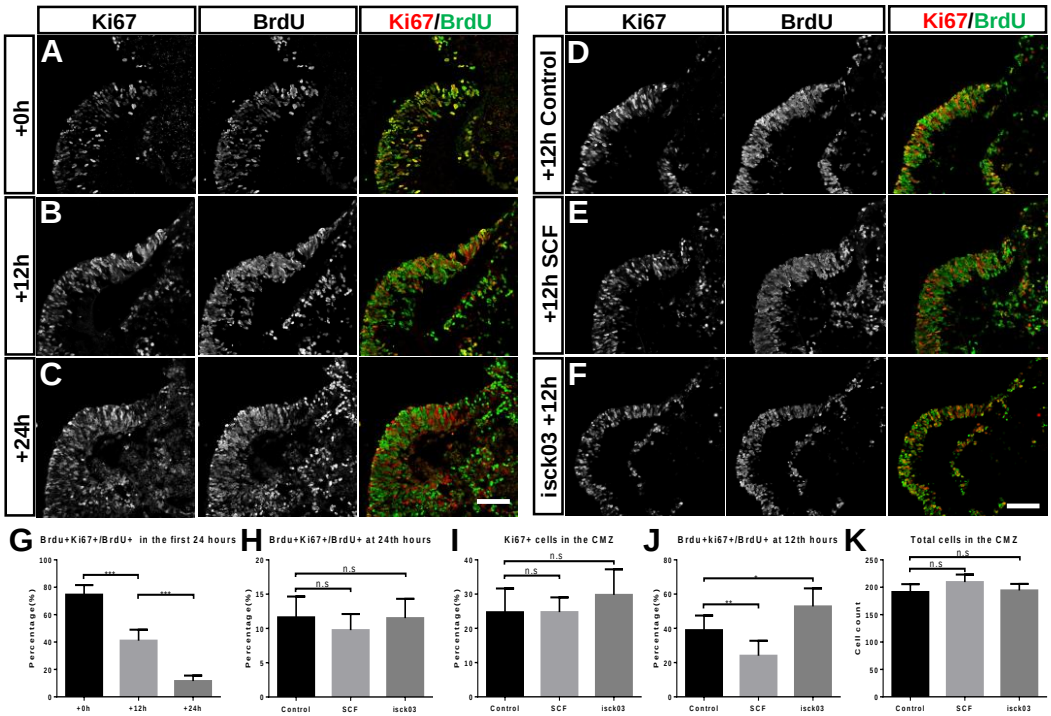

Figure S4. The SCF/SCFR signaling regulated the cell cycle progression of RPCs in the CMZ. **(A~C)** Double staining of BrdU and Ki67 at 0^{th}, 12^{th},24^{th} hours after 24 hours BrdU incorporation. **(D~F)** Double staining of BrdU and Ki67 at an intermediate stage of cell cycle(12^{th} hours after 24 hours BrdU incorporation) in the control, SCF, and isck03 treatment group. **(G)** The percentage of $BrdU^+/Ki67^+$ cells in the $BrdU^+$ cells at different stage in the first 24 hours after BrdU incorporation. **(H)** The percentage of $BrdU^+/Ki67^+$ cells in the $BrdU^+$ cells at the 24^{th} hours after BrdU incorporation. **(I)** The percentage of $Ki67^+$ cells in the total cells of CMZ after 24 hours of SCF or isck03 treatment. **(J)** The percentage of $BrdU^+/Ki67^+$ cells in the $BrdU^+$ cells at the 12^{th} hours after BrdU incorporation. **(K)** The cell count of all the cells in the CMZ at D30 after SCF or isck03 treatment. Data are shown as mean±SEM. Independent sample t test.
*p < 0.05, **p < 0.01; ***p < 0.005, ****p < 0.0001. Scale bars: 200 μm (A~F).

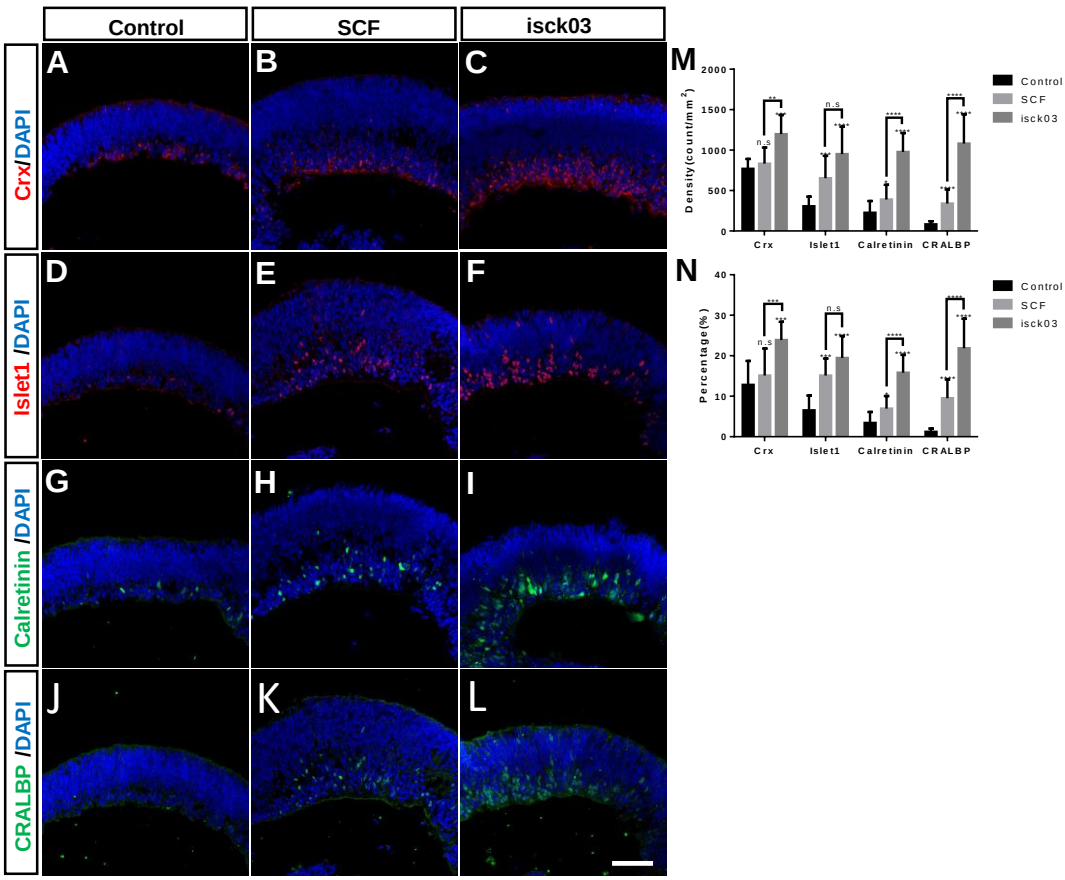

Figure S5. Imbalance of the SCF/SCFR signaling disturbed the early neurogenesis of NR. **(A~L)** Immunostaining of each cell type progenitor marker in the control, SCF, and isck03 group at D30: Crx (A~C, red), Islet1 (D~F, red), Calretinin (G~I, green), CRALBP (J~L, green). **M~N,** Statistics analysis of cell density and cell percent of each committed progenitor cells among the control, SCF, and isck03 group. Data are shown as mean ±SD. Independent sample t test. *p < 0.05, **p < 0.01; ***p < 0.005, ****p < 0.0001. Scale bars: 100 μm (A~L).

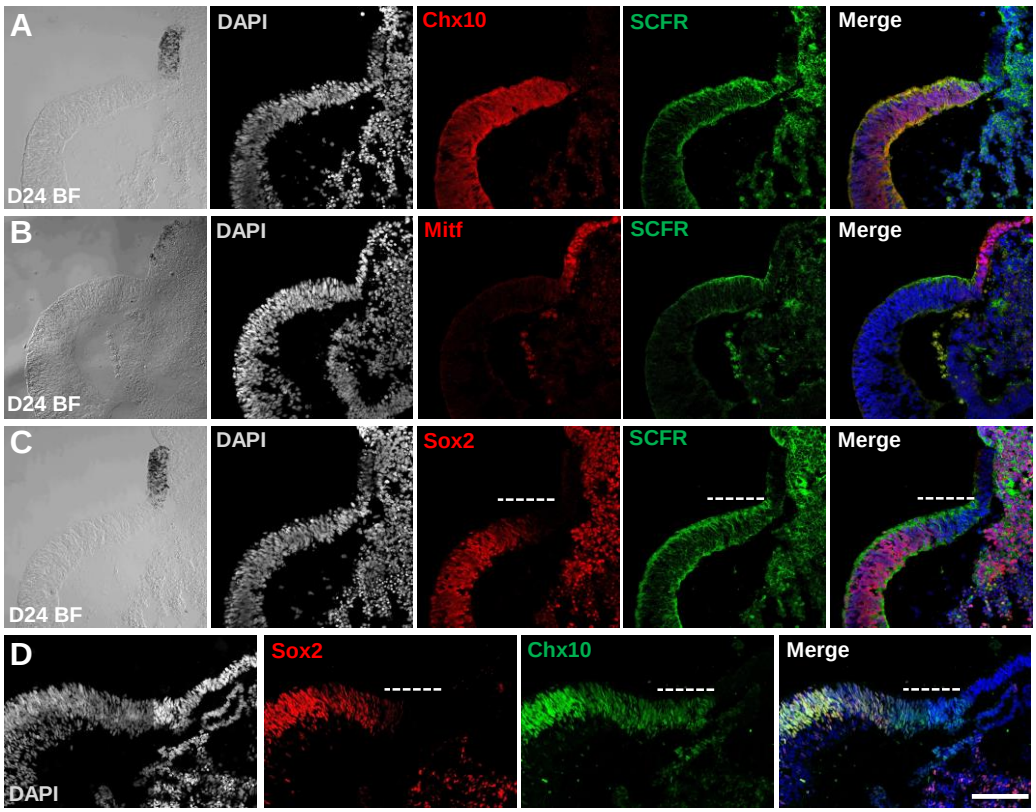

Figure S6. The heterogeneity of cells in the CMZ of human OC.
(A~C) Immunostaining of D24 retinal organoid shows overlapped distribution of SCFR and Chx10(A); distinctive distribution of SCFR and MITF(B); similar distribution of SCFR and Sox2(C). **(D)** Double staining of Chx10 and Sox2 at D24 shows the cell heterogeneity in the CMZ(dashed line: $Chx10^{+}Sox2^{-}$ region). Scale bars: 200 μm(A~D).