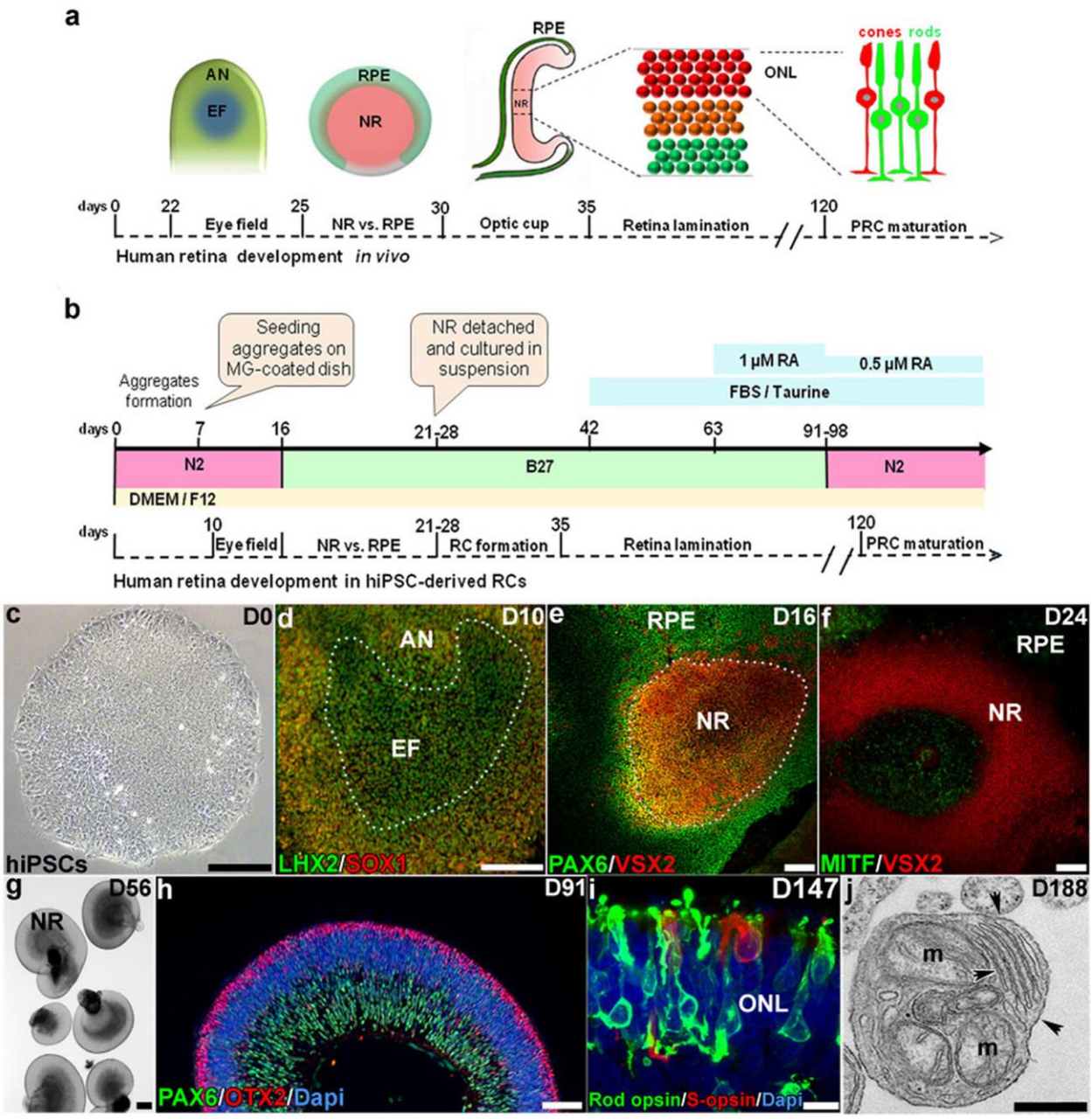
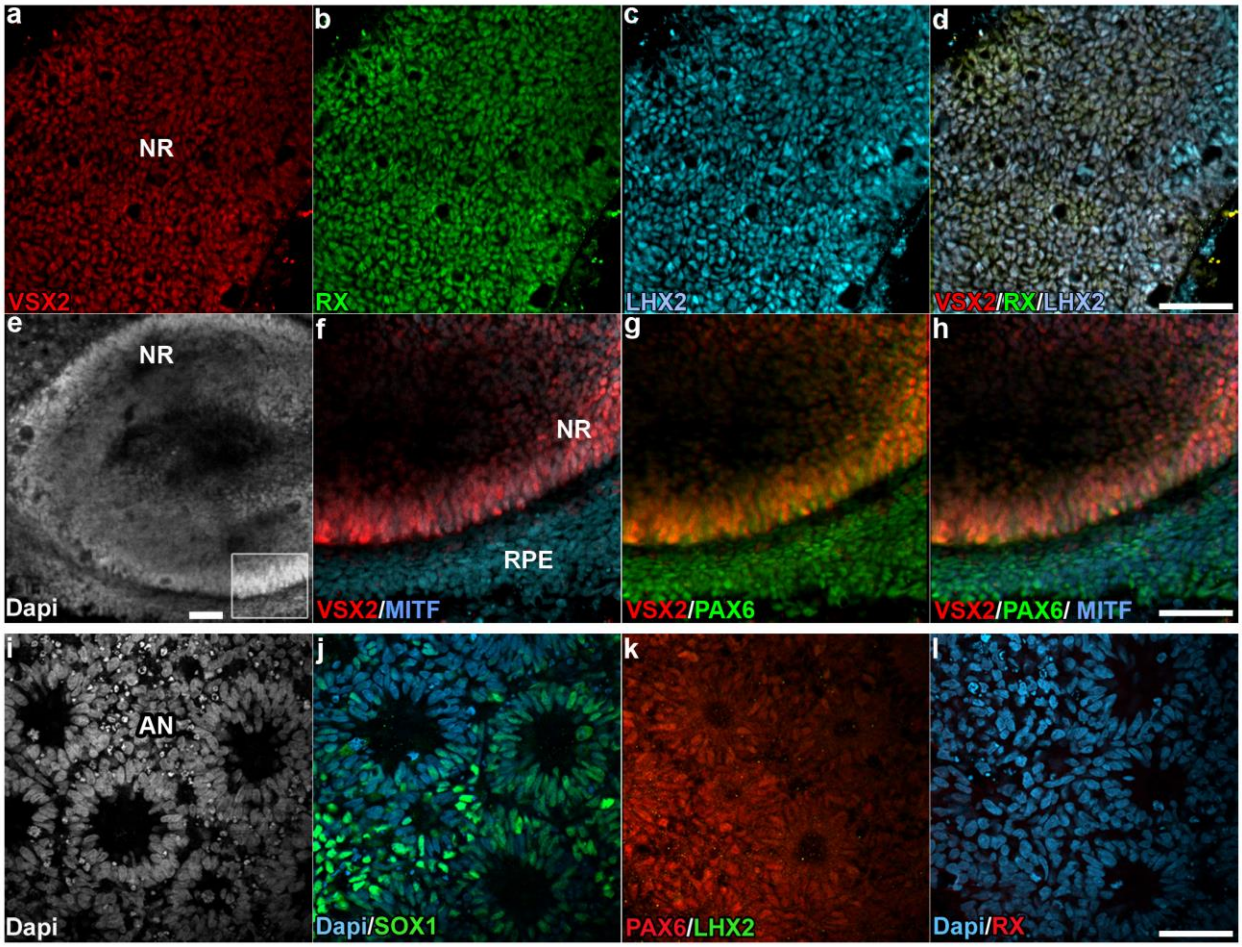
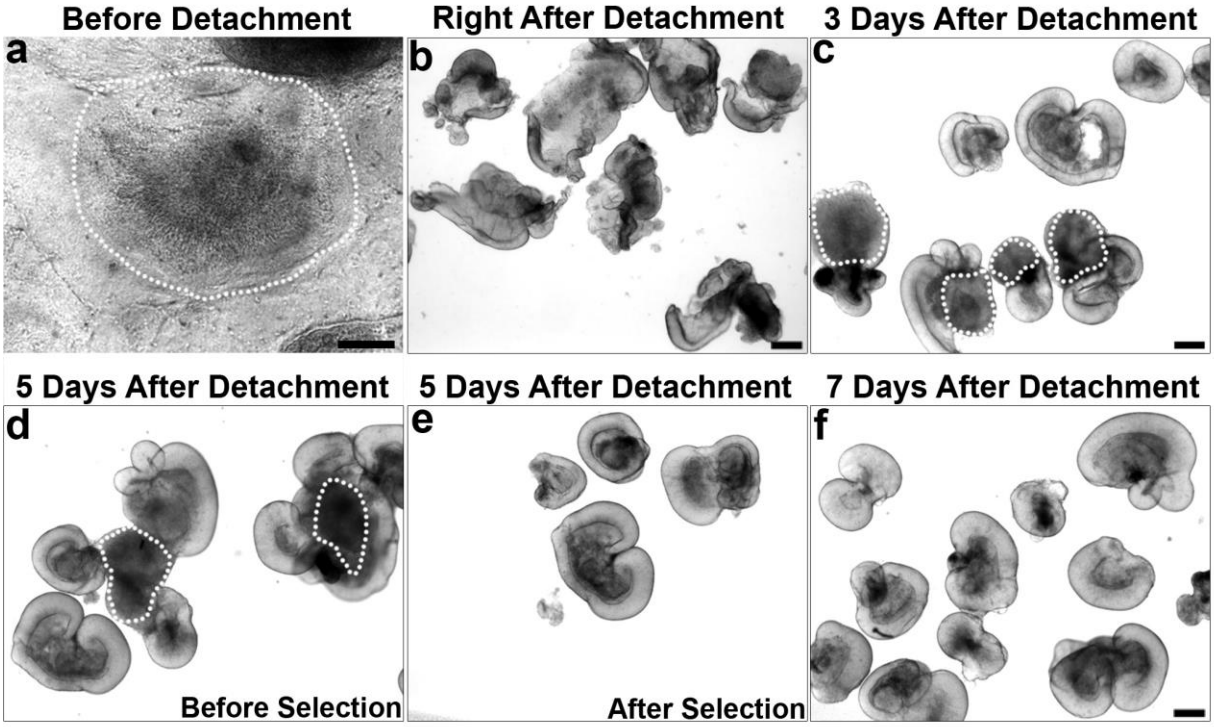


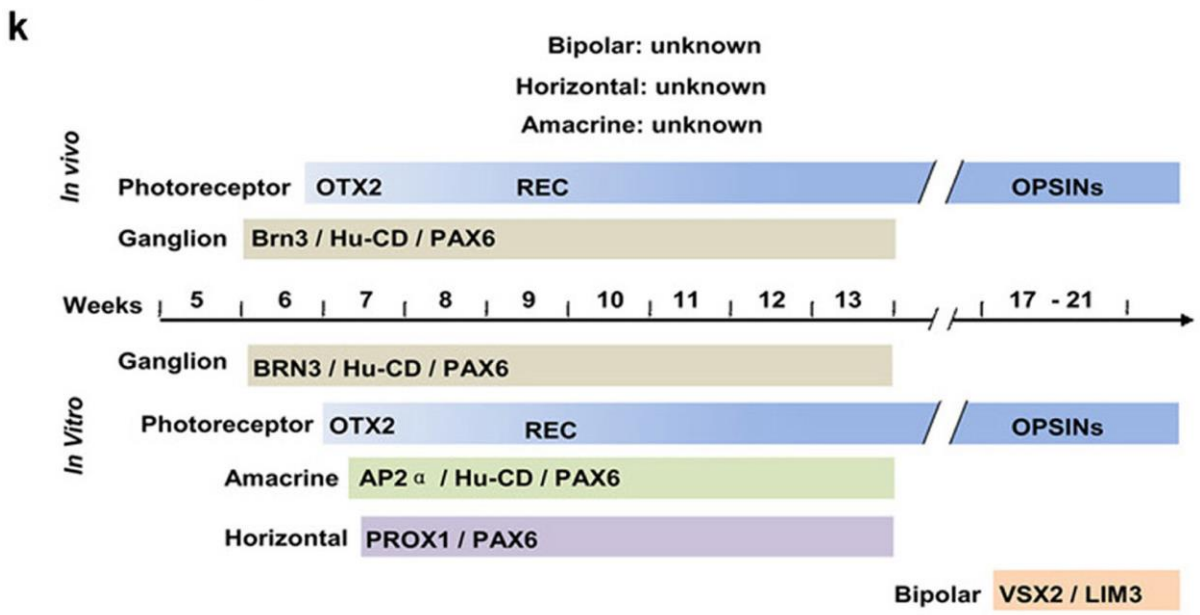
Supplementary Figure 1. Retinogenesis during human embryonic development and in 3-D retinal cups (RCs) derived from hiPSC. a, Main steps leading to the formation of the human retina *in vivo*. **b**, Diagram of the optimized culture conditions used for inducing hiPSC to recapitulate retinogenesis *in vitro*. **c-j**, Main steps of RC development *in vitro*: hiPSC (c) differentiated into retinal progenitors that self-organized into eye field-like domains (EF, d; AN: anterior neuroepithelium) which subsequently differentiated into a central neural retina (NR) domain and a peripheral retinal pigment epithelium (RPE) domain (e). As differentiation progressed, the NR domain acquired an optic-cup-like shape (f) and formed a 3-D RC when cultured in suspension (g). Over time, RCs acquired the characteristic retinal lamination (h), including a well-organized outer nuclear layer (ONL) containing highly mature rod and cone photoreceptors showing expression of the corresponding opsins (i) and formation of outer-segment discs (demarcated by arrowheads, j; m: mitochondrion). Scale bars: 200µm (c and g); 100µm (d-f and h); 20µm (i); 0.5µm (j).



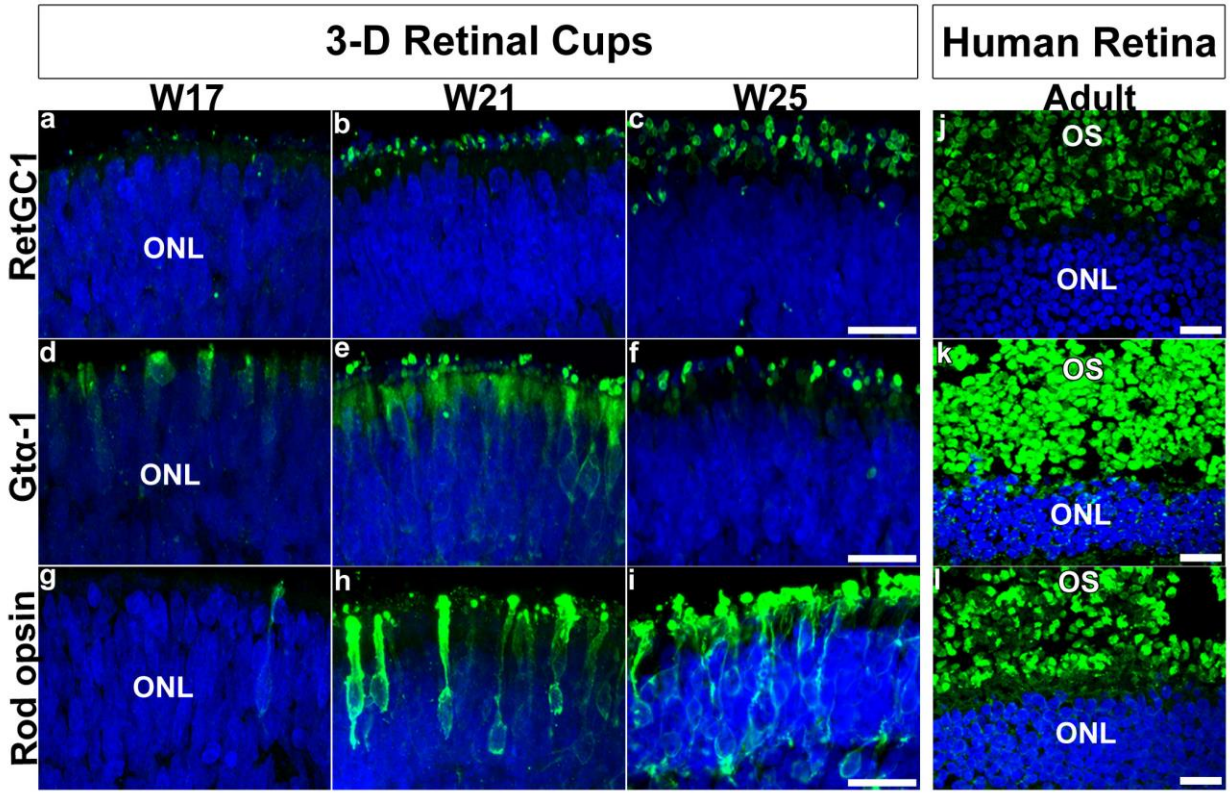
Supplementary Figure 2. Coexisting cellular domains in D16 to D28 cultures. Cell differentiation in these cultures is not synchronized; therefore, in a given culture dish, early neural retina (NR) domains (a-d), optic-cup-like NR and surrounding retinal pigment epithelium (RPE) domains (e-h) and anterior neuroepithelial (AN) cells forming characteristic rosettes (i-l) were found simultaneously. Scale bars: 50µm.



Supplementary Figure 3. Progressive formation of 3-D retinal cups (RCs) from detached neural retina (NR) domains. a, a NR domain from a D26 culture before detachment. **b**, several NR domains right after being manually detached and switched to culture in suspension. **c-f**, under suspension culture, neural retinal epithelia within the detached NR domains increased in size and thickness and formed RCs. Some RCs grew attached to non-retinal cellular aggregates (demarcated by dashed lines in c and d) which appeared darker and disorganized. For long-term culture, RCs were separated from non-retinal cellular aggregates (e,f). Scale bar: 100μm



Supplementary Figure 4. Timeline of retinal neurogenesis in 3-D RCs *in vitro* compared to *in vivo* human retina. The genesis of the different retinal neuronal precursors in our 3-D RCs followed the sequential pattern characteristic of the vertebrate retina[41,42]. The timing of appearance of ganglion and photoreceptor precursors as well as the onset of expression of their cell-type-specific proteins coincided with that observed in the human embryonic retina[44,47,48,69,70]. The specific timing of amacrine, horizontal and bipolar cell differentiation in the developing human retina is still unknown.



Supplementary Figure 5. hiPSC-derived photoreceptors within the retinal cups (RCs) gradually acquired expression of proteins involved in phototransduction. a-i, As observed in the developing human retina *in vivo*[44], expression of the phototransduction proteins increased in parallel to that of rod opsin. **j-l,** Antibodies used for detecting proteins associated with the phototransduction pathway in the RCs were validated in adult human retina as shown in these examples. Scale bars: 20µm.

Supplementary Table 1. Human iPSC lines used in this study.

Cell Line	Cell of Origin	Reprogramming Method	Genomic Integration	Ref
CB-iPSC6.2	Human $CD34^+$ cord blood-derived myeloid progenitors	non-viral 7-factor episomes (SOX2, OCT4, KLF4, MYC, NANOG, LIN28, SV40T) with BMSC-priming of myeloid donors	NO	38
KA.1	Human adult keratinocytes	non-viral 7-factor episomes (SOX2, OCT4, KLF4, MYC, NANOG, LIN28, SV40T)	NO	39,66
IMR90-4	Human fibroblast cell line IMR90	Lentiviral 4 factors (SOX2, OCT4, NANOG, LIN28)	YES	37

Three representative human iPSC lines of different cell origin and reprogramming method were chosen to test the reproducibility of our method across cell lines.