Generation of *RB1*$^{-/-}$ RUCDRi002-A-65 iPSC line

The healthy control human iPSC line, RUCDRi002-A (Baghbaderani *et al.,* 2015) was used as the parental stem cell source to generate *RB1*$^{-/-}$ knockout lines. Guide RNAs targeting the exon 18 of the h*RB1* were designed using CHOPCHOP (https://chopchop.cbu.uib.no/). The sense and anti-sense guide oligos were annealed *in vitro* and cloned into the BsmBI site of the lentiCRISPRv2 construct (Addgene plasmid #52961). The RB1-Ex18-CS1 gRNA target region sequence is 5'AAACAATCAAAGGACCGAGA3'. The guide encoding plasmid construct was then transfected into the parental hiPSCs at 50-60% confluency, using the Lipofectamine™ Stem Transfection Reagent (Invitrogen Cat. No# STEM00001), according to the manufacturers' instructions. The transfected cells are further selected using 750 ng/mL Puromycin for 24 h, and the surviving cell pool was then clonally expanded. Single cell clones were manually picked and clonally expanded for genotyping. Genomic DNA was isolated and used as templates for target region-specific PCR using the forward primer: 5'GCCACTGTCAATTGTGCCTAAAATTC3' and the reverse primer: 5'GTGATTCAGTAGCACTCTGTACTC3' and the amplicons were subjected to Sanger sequencing to confirm the presence of edits. In case of compound heterozygous edits, PCR products of individual clones were separately cloned into pMOS-Blue vector and the inserts were sequenced to confirm the two different allelic in-dels and the edit margins. Clones with confirmed edits were maintained in Essential 8™ medium (Thermo Fisher Scientific) at 37°C with 5% CO_2 supply and were passaged at regular intervals in 1:6 split ratios using cell dissociation solution (0.5 mM EDTA and 30 mM NaCl in 1X DPBS) and rock inhibitor treatment. Passage 10 cells were characterized for their stemness, pluripotency and genomic integrity.