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Control of Microbial Opsin Expression in Stem Cell Derived Cones for Improved Outcomes in Cell Therapy.

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Human-induced pluripotent stem cell (hiPSC) derived organoids have become increasingly used systems allowing 3D-modeling of human organ development, and disease. They are also a reliable source of cells for transplantation in cell therapy and an excellent model to validate gene therapies. To make full use of these systems, a toolkit of genetic modification techniques is necessary to control their activity in line with the downstream application. We have previously described adeno-associated viruse (AAV) vectors for efficient targeting of cells within human retinal organoids. Here, we describe biological restriction and enhanced gene expression in cone cells of such organoids thanks to the use of a 1.7-kb L-opsin promoter. We illustrate the usefulness of implementing such a promoter to enhance the expression of the red-shifted opsin Jaws in fusion with a fluorescent reporter gene, enabling cell sorting to enrich the desired cell population. Increased Jaws expression after transplantation improved light responses promising better therapeutic outcomes in a cell therapy setting. Our results point to the importance of promoter activity in restricting, improving, and controlling the kinetics of transgene expression during the maturation of hiPSC retinal derivatives. Differentiation requires mechanisms to initiate specific transcriptional changes and to reinforce those changes when mature cell states are reached. By employing a cell-type-specific promoter we put transgene expression under the new transcriptional program of mature cells.

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Conflict of interest statement: MG-H, AC, OG, DD, JD, and J-AS are inventors on a patent on the use of hiPSC to treat retinal degeneration (WO2018055131). DD is an inventor on a patent of adeno-associated virus virions with variant capsid and methods of use thereof with royalties paid to Avalanche Biotech (WO2012145601 A2). DD and HK are inventors on pending patent applications on noninvasive methods to target cone photoreceptors (EP17306429.6 and EP17306430.4) licensed to Gamut Tx. DD, HK and OG are founders of Gamut Tx. OG and J-AS are inventors on a patent on hiPSC retinal differentiation (WO2014174492 A1). J-AS is a founder and consultant for Pixium Vision and GenSight Biologics and is a consultant for Sanofi-Fovea, Genesignal, and Vision Medicines. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interes