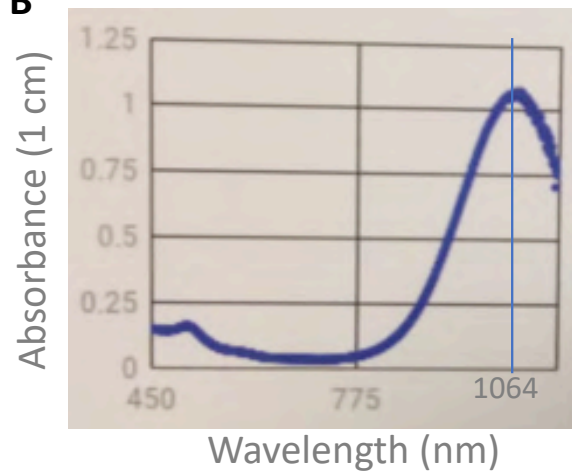


OMTM, Volume 17

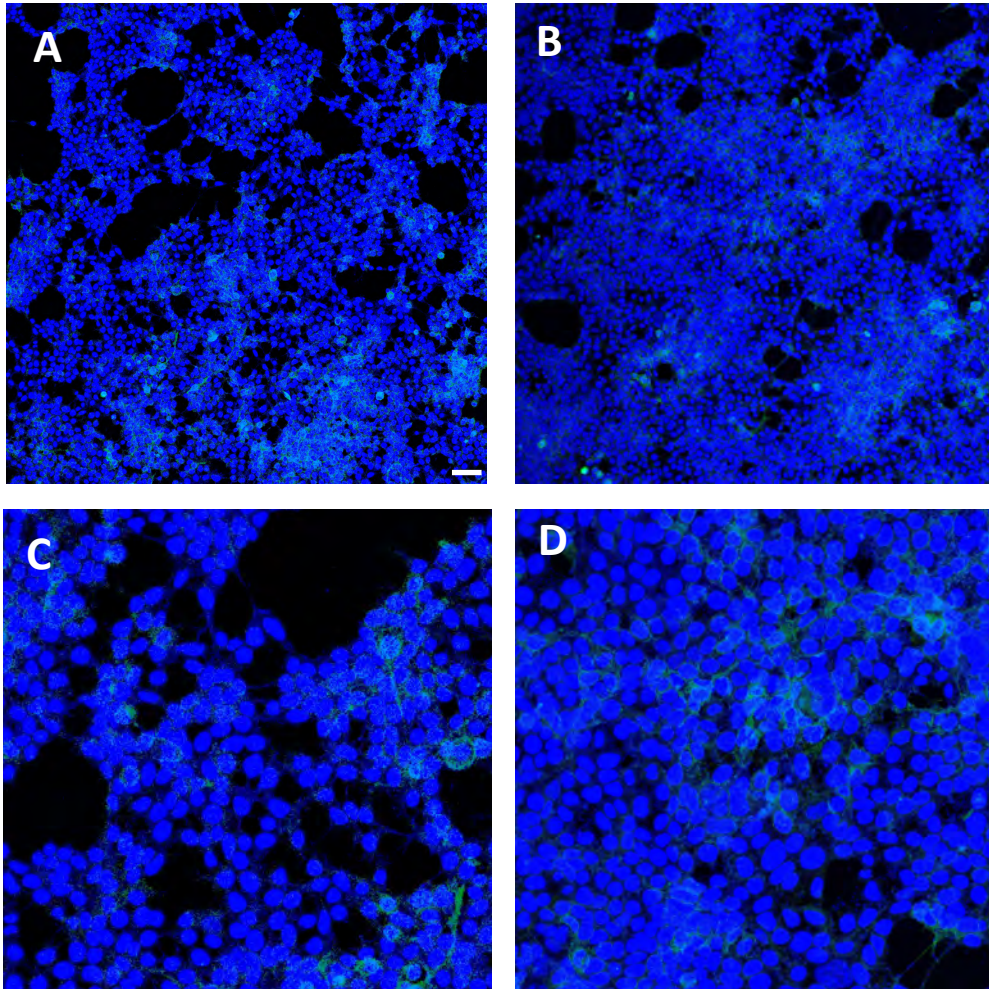
Supplemental Information

**Near-Infrared Laser-Based Spatially
Targeted Nano-enhanced Optical Delivery
of Therapeutic Genes to Degenerated Retina**

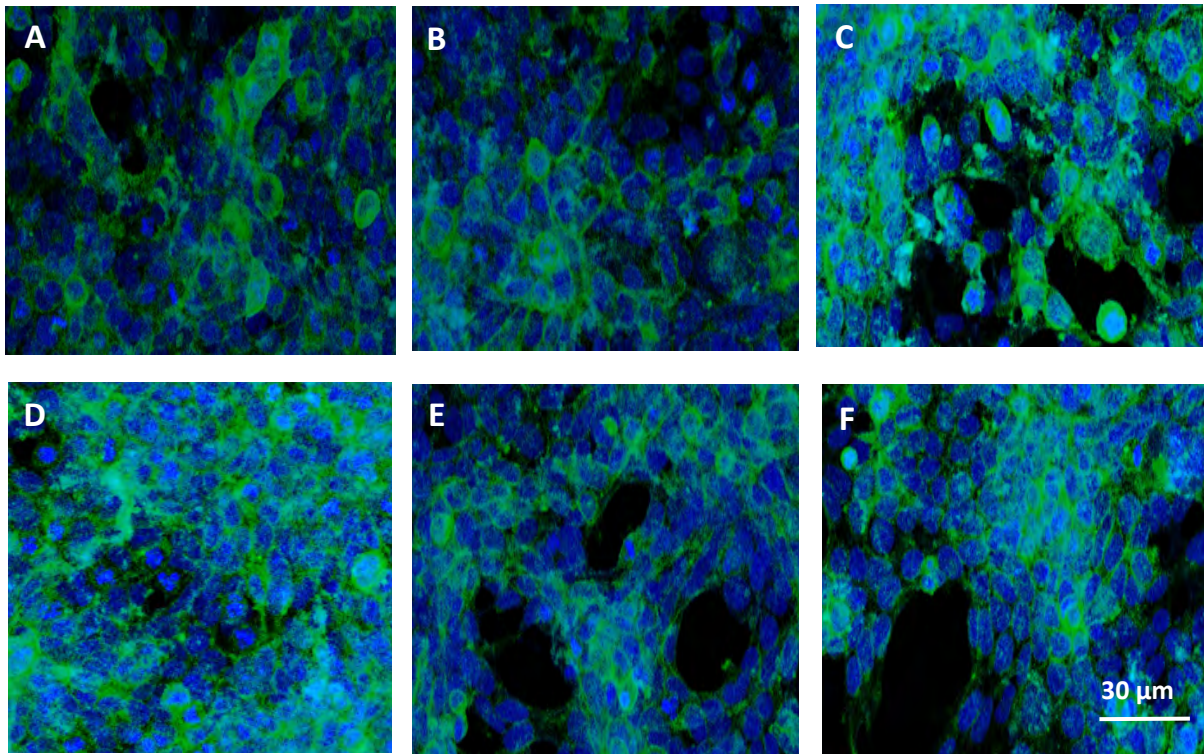
Subrata Batabyal, Sivakumar Gajjerman, Kissaou Tchedre, Adnan Dibas, Weldon Wright, and Samarendra Mohanty

A**B**

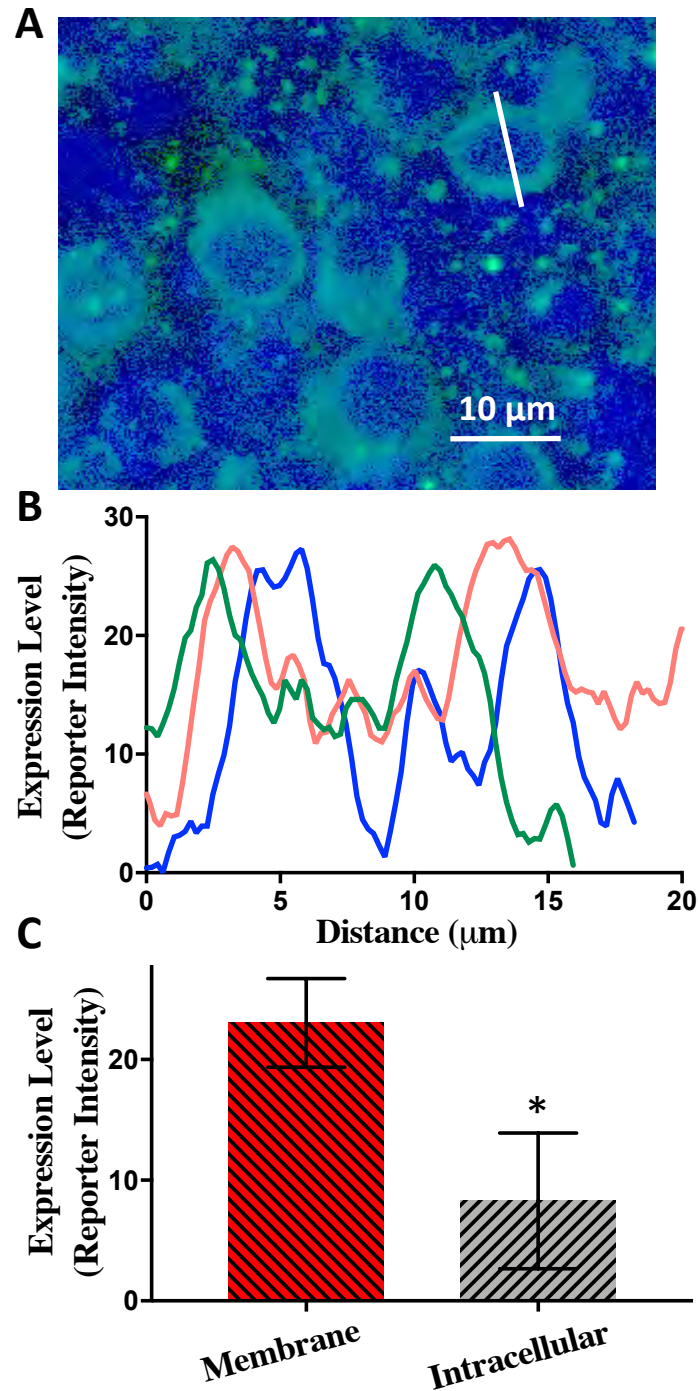
Suppl. Fig. 1. Structural and spectroscopic properties of gold nanorods. (A) Transmission electron microscopic image of gold nanorods used for nano enhanced optical delivery of CAG-MCO-II-mCherry into cells. These nanorods are functionalized with Concavalin A for binding of membrane of the cells. (B) Optical density of the gold nanorods, showing a peak at 1064 nm.



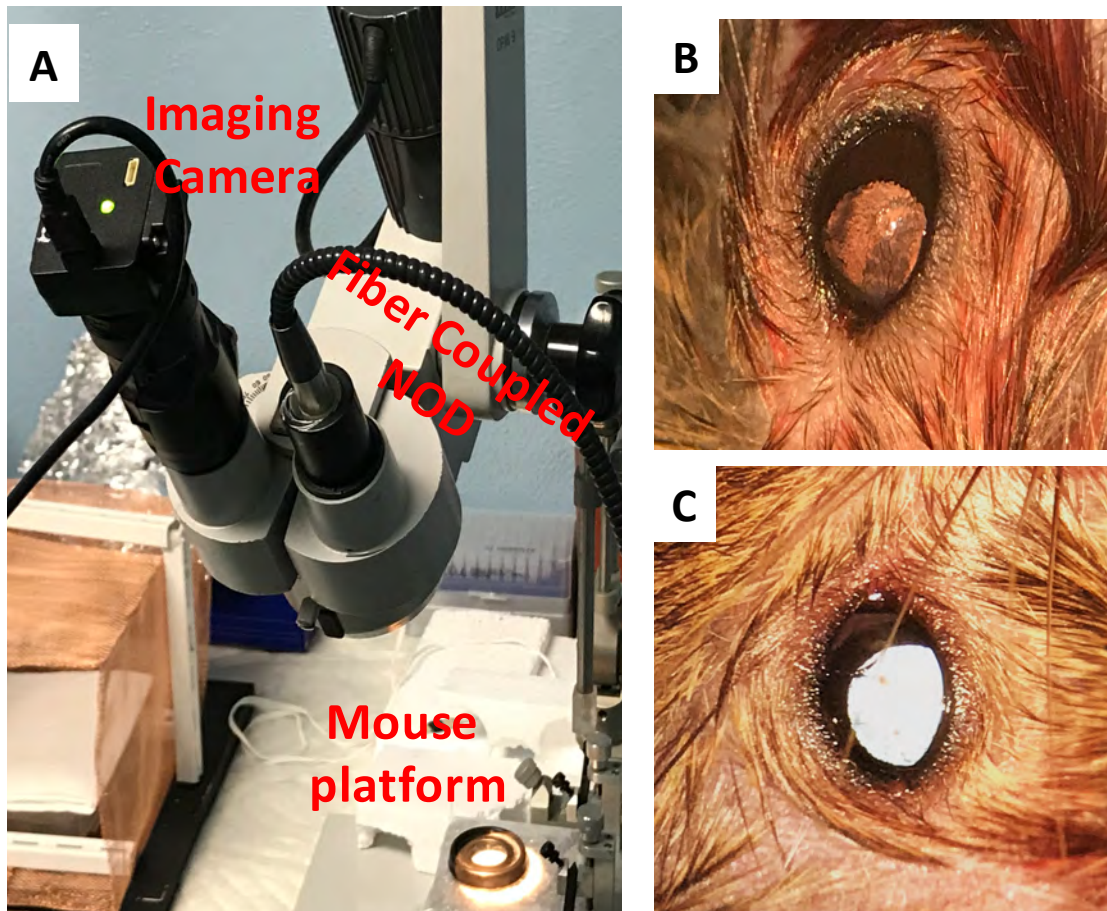
Suppl. Fig. 2. Confocal imaging of immunostained HEK cells for various experimental controls. (A) -Plasmid, - fGNR, - Laser; (B) + plasmids, + fGNRs - Laser. (C, D) 3x zoomed image of panels A and B, respectively. Primary antibody: Anti-mCherry, Secondary: Alexa 488. Nuclear stain: DAPI (blue). Scale bar = 50 μm .



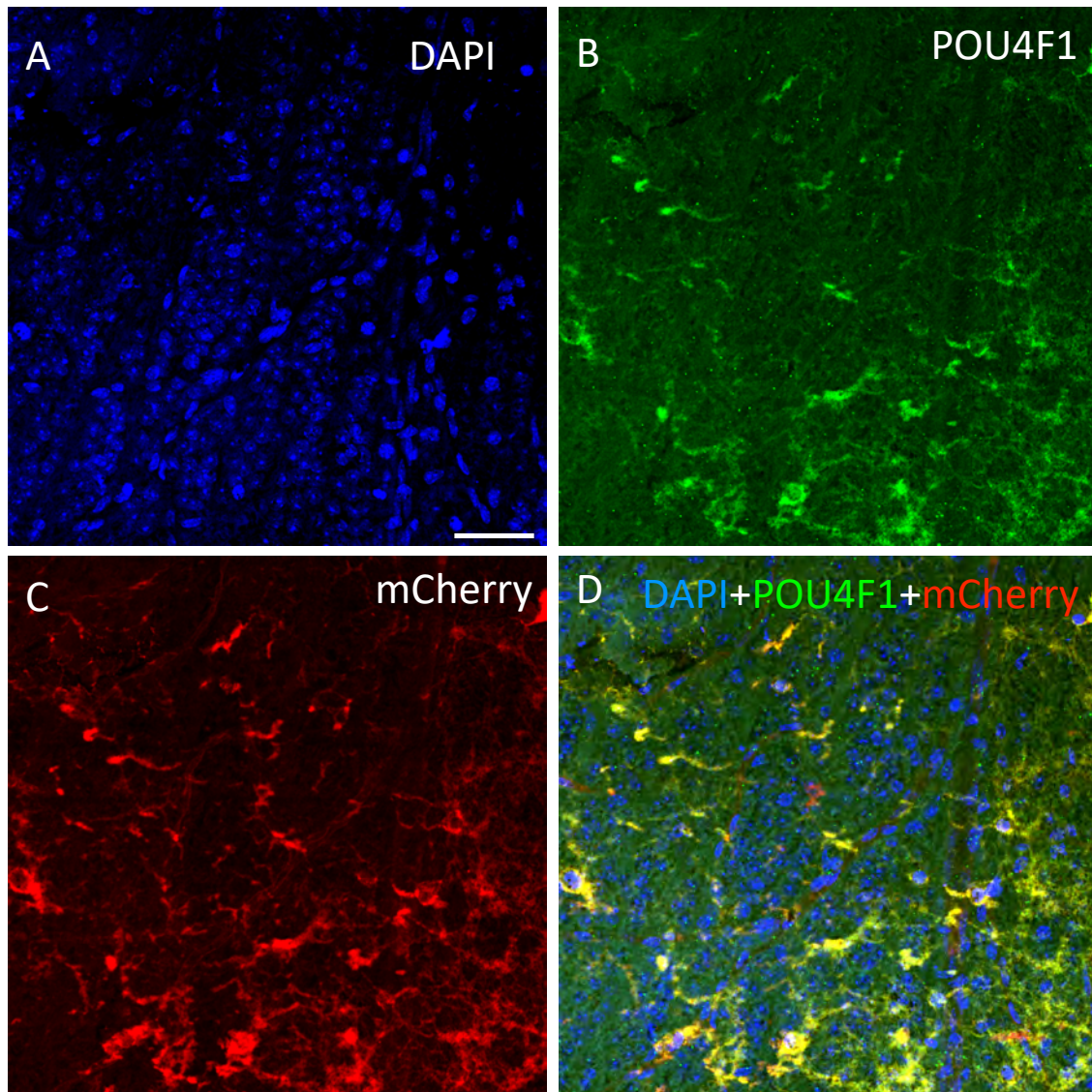
Suppl. Fig. 3. NOD treated HEK cells using 1064 nm Nanosecond laser beam with different pulse energy and exposure. (A) 2.5 mJ/mm² (10 mJ/pulse, 10 Hz, 5 s); (B) 5 mJ/mm² (20 mJ/pulse, 10 Hz, 5 s); (C) 7.5 mJ/mm² (30 mJ/pulse, 10 Hz, 5 s); (D) 5 mJ/mm² (10 mJ/pulse, 10 Hz, 10 s); (E) 10 mJ/mm² (20 mJ/pulse, 10 Hz, 10 s); and (F) 15 mJ/mm² (30 mJ/pulse, 10 Hz, 10 s). Energy density calculated using laser exposure area of 200 mm².



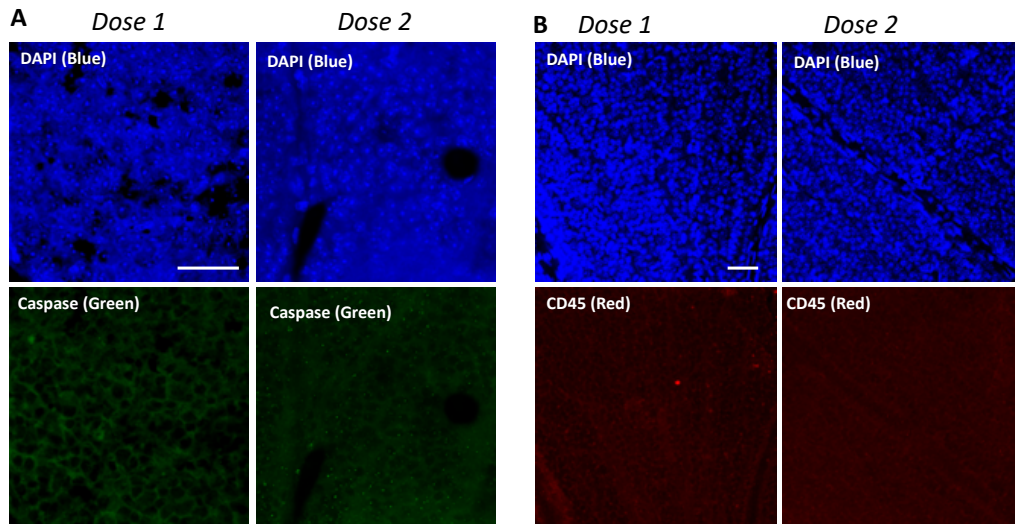
Suppl. Fig. 4. Cell specific expression in retina explant after nanosecond pulsed laser beam based NOD. (A) Retinal cells showing the expression of MCO-II-mCherry (Immunostained) after spatially targeted NOD (laser dose: 5 mJ/mm²). Primary antibody: Anti-mCherry, Secondary: Alexa 488. Nuclear stain: DAPI (blue). (B) Reporter fluorescence intensity along lines drawn across the transfected cells. (C) Quantitative comparison of mCherry expression in membrane and cytoplasm. Mean ± S. D. *p<0.05.



Suppl. Fig. 5. Set up for *in-vivo* NOD using 1064 nm continuous wave laser beam. (A) Picture of the set up, showing fiber-coupled laser beam and imaging camera integrated with a surgical microscope. (B) Image of mouse eye dilated with Tropicamide to allow NOD laser beam irradiation of retina. (C) Image of mouse retina being irradiated by the near-infrared NOD laser beam.



Suppl. Fig. 6. *In-vivo* optical delivery of MCO-II to *rd10* mice retina is Retinal Ganglion Cell specific. Fluorescence image of retina stained with: (A) nuclear stain DAPI. (B) RGC-specific staining using POU4F1. (C) Fluorescence image of reporter-mCherry expressed in retina after *in-vivo* optical delivery using CW 1064 nm laser beam. (D) Composite image of DAPI (blue), POU4F1 (green) and mCherry (red), showing co-localization of RGC with mCherry expression (yellow color). Scale bar = 50 μ m.



Suppl. Fig. 7. *In-vivo* optical delivery of MCO-II to *rd10* mice retina does not compromise viability of retina or elicit immune response. (A) Representative fluorescence images of retina stained with Caspase-3 (green) and DAPI (blue) for two different cw laser doses. Dose 1: 1.8 J/mm² (30 mW for 60 s); Dose 2: 3.6 J/mm² (60 mW for 60 s). Non-detectable apoptotic cells after optical delivery. (B) No detectable CD45 (red) signal, suggesting the absence of immune cells after MCO transfection. Dose 1: 0.9 J/mm² (30 mW for 30 s); Dose 2: 1.8 J/mm² (30 mW for 60 s). Laser irradiation area: 1 mm². Scale bar = 50 μm.