

Comprehensive transcriptomic analysis reveals canonical and novel pathways modulated by nanoceria in mammalian retinal degeneration

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Abstract

Retinal neurodegenerative diseases such as Age-related Macular Degeneration (AMD) and Retinitis Pigmentosa cause irreversible vision loss due to the limited regenerative capacity of the mammalian retina. Cerium oxide nanoparticles (nanoceria) are emerging therapeutics against oxidative stress and inflammation, major drivers of photoreceptor degeneration, but their mechanisms remain incompletely understood. We performed retinal transcriptomic analysis in a rat AMD model induced by intense light and treated intravitreally with nanoceria. Six groups were analyzed: control, light damage, vehicle, nanoceria, vehicle + light damage, and nanoceria + light damage. Light damage activated inflammatory and apoptotic programs, with upregulation of cytokines (*Tnf*, *Il6*, *Il1b*, *Ccl2*) and downregulation of photoreceptor genes (*Rho*, *Pde6a/b*, *Gnat1*). Nanoceria treatment counteracted these effects, suppressing pro-inflammatory mediators, restoring antioxidative genes (*Nfe2l2*, *Gclc*, *Sod2*), and enhancing neuroprotective factors (*Bdnf*, *Cntf*, *Ngf*). Pathway analyses revealed inhibition of TNF/NF- κ B/IL-17 signaling and activation of PI3K-Akt, JAK-STAT, and neurotrophin pathways. Unexpectedly, nanoceria also modulated amino acid and insulin metabolism (*Ass1*, *Cps1*, *Insr*, *Irs1*, *Slc2a4*) and reactivated transcription factors (*Ascl1*, *Sox2*, *Notch1*) typically silent in adult retina. Our findings highlight nanoceria as a multifunctional therapeutic that mitigates retinal degeneration by coordinating oxidative, inflammatory, and regenerative responses, supporting its translational potential to preserve vision in retinal neurodegenerative disease.

1. Introduction

Retinal neurodegenerative diseases represent a leading cause of blindness worldwide, with age-related macular degeneration (AMD) and retinitis pigmentosa (RP) affecting millions of patients ¹. Unlike lower vertebrates such as zebrafish that possess remarkable retinal regenerative capacity through Müller glia reprogramming, the adult mammalian retina has minimal intrinsic regenerative potential, making photoreceptors loss irreversible ². The pathophysiology of retinal degeneration involves complex interactions between oxidative stress, chronic inflammation, and metabolic dysfunction ^{3,4}. Acute high-intensity light damage (LD) in rodents reproduces key features of photoreceptor degeneration observed in AMD, driven by oxidative stress, inflammatory activation, and apoptosis ³⁻⁵. These models provide a tractable platform to dissect disease mechanisms and evaluate candidate neuroprotective interventions.

Cerium oxide nanoparticles (nanoceria) have emerged as promising therapeutics due to their unique redox-switching properties between Ce^{3+}/Ce^{4+} oxidation states, conferring catalytic, self-regenerating free-radical scavenging activity ^{6,7}. Previous studies demonstrated that intravitreal nanoceria reduce the ROS accumulation, temper microgliosis, preserve photoreceptors, and protect the retinal pigment epithelium in LD paradigms ^{3,4,6-9}. However, the genome-wide molecular programs engaged by nanoceria in the degenerating mammalian retina remain incompletely defined.

Here we perform bulk RNA-seq in a rat LD model with and without intravitreal nanoceria to systematically map nanoceria-responsive gene networks. We test whether nanoceria not only blunt

canonical oxidative-stress and inflammatory pathways but also modulate metabolic and regeneration-linked circuits relevant to photoreceptor survival. By integrating differential expression and pathway enrichment analyses, we provide a transcriptomic framework for nanoceria's mode of action and nominate testable targets for neuroprotective therapy in AMD-like injury ⁵.

2. Results

2.1. Light damage activates inflammatory/apoptotic programs and suppresses photoreceptor identity

Acute high-intensity light exposure (LD) produced a broad transcriptional shift versus controls (CTRL), with hundreds of DEGs (e.g., ~ 920 at FDR < 0.05; 666 up/254 down). The violin plots highlight a clear separation between experimental groups, with nanoceria-treated samples (NANO) distinctly clustering away from both vehicle (VEH) and control (CTRL) groups. This segregation in the PCA space indicates a robust transcriptional shift modulated by nanoceria treatment, suggesting enhanced neuroprotection and antioxidative responses (Fig. 1).

Upregulated genes encompassed cytokines/chemokines (*Tnf*, *Il6*, *Il1b*, *Ccl2/Ccl3/Ccl4*), gliosis/microglial markers (*Gfap*, *Aif1*), and stress/apoptosis mediators (Bax, Casp3), while photoreceptor and visual-cycle transcripts were prominently reduced (*Rho*, *Pde6a/b*, *Gnat1*, *Cnga1*; transcriptional regulators *Crx*, *Nrl*, *Nr2e3*). Enrichment analyses highlighted TNF/NF-κB and p53/apoptosis among upregulated pathways, with strong depletion of phototransduction and retinol/visual-cycle terms. These data establish the canonical oxidative-inflammatory injury signature and loss of photoreceptor identity in LD (Fig. 2; Table 1; Fig. S1).

Table 1

Consolidated “Top DEGs” for the two pivotal contrasts. Top up- and down-regulated genes for LD vs CTRL and NANO-LD vs LD (ranked by $|\log_2FC|$; FDR < 0.05). Complete DEG lists for all five contrasts are provided in Supplementary Tables S1-S5.

<i>LD vs CTRL – Top up-regulated (left) and down-regulated (right)</i>					
Gene	$\log_2FC$	FDR	Gene	$\log_2FC$	FDR
<i>Hprt1</i>	8.95	4.2e – 03	<i>Rn60_20_0047.3</i>	–8.29	6.6e – 05
<i>Hsp90aa1</i>	7.97	1.6e – 02	<i>RGD1560883_2</i>	–7.98	1.1e – 02
<i>LOC108348074</i>	7.82	6.9e – 03	<i>Matn1</i>	–7.57	5.4e – 03
<i>Fam71a</i>	7.42	7.3e – 08	<i>ENSRNOG00000046377</i>	–7.35	1.8e – 02
<i>Olr1641</i>	7.29	5.9e – 03	<i>Gdf1</i>	–7.34	3.2e – 07
<i>Haus1</i>	7.17	2.0e – 02	<i>Ndufa10l1</i>	–7.26	2.5e – 02
<i>Il6</i>	7.07	2.5e – 18	<i>LOC108348155</i>	–7.20	1.2e – 02
<i>Plscr5</i>	6.74	9.3e – 03	<i>RGD1564463</i>	–7.15	7.7e – 03
<i>Ccl2</i>	6.68	7.6e – 113	<i>Bpifa6</i>	–6.99	1.1e – 06
<i>ENSRNOG00000037495</i>	6.63	7.1e – 04	<i>LOC100910708</i>	–6.71	1.1e – 02
<i>NANO-LD vs LD – Top up-regulated (left) and down-regulated (right)</i>					
Gene	$\log_2FC$	FDR	Gene	$\log_2FC$	FDR
<i>Yme1l1</i>	8.24	1.4e – 02	<i>LOC100909505</i>	–10.51	2.3e – 03
<i>AABR07065782.1</i>	7.52	2.9e – 02	<i>LOC100909505 (iso)</i>	–9.38	2.7e – 03
<i>AABR07060963.1</i>	7.16	1.3e – 02	<i>Ccl2</i>	–7.17	2.0e – 02
<i>Ngf</i>	6.88	3.8e – 02	<i>Cd14</i>	–6.62	3.3e – 02
<i>Bdnf</i>	6.70	4.9e – 02	<i>Il6</i>	–6.50	2.9e – 02
<i>Sirt1</i>	6.55	3.2e – 02	<i>C1qb</i>	–6.33	3.5e – 03
<i>Mt1</i>	6.33	1.8e – 02	<i>Tnf</i>	–6.12	4.8e – 02
<i>LOC689527</i>	6.25	4.5e – 02	<i>Nfkbia</i>	–5.90	4.4e – 02
<i>Nfe2l2 (Nrf2)</i>	6.10	3.1e – 02	<i>Serpine1</i>	–5.80	4.6e – 02
<i>Cntf</i>	5.98	2.4e – 02	<i>Lyz2</i>	–5.75	2.2e – 02

2.2. Intravitreal vehicle injection exerts minimal transcriptomic impact

Vehicle (VEH) alone induced only minor changes relative to CTRL (dozens of DEGs with small effect sizes and no significant GO/KEGG categories after correction), indicating the injection procedure/vehicle is largely transcriptionally inert at the assayed time point. Similarly, VEH-LD vs LD showed only subtle and inconsistent differences, confirming that injection does not confound the LD injury signature (Supplementary Fig. S2–S3; Supplementary Tables S3–S4).

2.3. In uninjured retina, nanoceria prime a cytoprotective mitochondrial/antioxidant state

Nanoceria treatment in healthy eyes (NANO vs CTRL) reprogrammed the transcriptome toward oxidative-stress resilience and metabolic competence. Antioxidant master regulator *Nfe2l2* (*Nrf2*) and canonical targets (*Hmox1*, *Nqo1*, *Gclc*, *Sod2*, *Gpx* family, *Prdx1*) were induced, alongside mitochondrial/OXPHOS and bioenergetic genes (*Idh3a/b*, *Cpt1a*, *Acat2*, *Ppargc1a*). In parallel, inflammatory and apoptotic transcripts (*Tnf*, *Il1b*, *Nlrp3*, *Casp3*, *Bax*) were reduced. These data support a “preconditioning” effect whereby nanoceria elevate antioxidant capacity and mitochondrial organization while dampening basal inflammatory tone (Fig. S4–S5; Supplementary Table S4).

2.4. In injured retina, nanoceria reverse degeneration signatures and enhance survival/trophic programs

In LD eyes, nanoceria (NANO-LD) broadly opposed the injury signature relative to LD alone. Damage-induced inflammatory mediators and gliosis markers (e.g., *Tnf*, *Il6*, *Il1b*, *Ccl2*, *Nfkbia*, *Gfap*, *Aif1*) were strongly reduced, while photoreceptor transcripts (*Rho*, *Pde6b*) were preserved/partially restored. Nanoceria concomitantly induced neuroprotective and trophic factors (*Bdnf*, *Cntf*, *Ngf*, *Sirt1*) and regeneration-linked regulators (*Ascl1*, *Sox2*, *Notch1*, *Wnt2b*). Pathway analyses showed suppression of TNF/NF- κ B/IL-17 and activation of PI3K–Akt, JAK–STAT, neurotrophin signaling, with strengthened oxidative phosphorylation and mitochondrial organization (Fig. 3; Table 2; Supplementary Fig. S6, Supplementary Table S5). Direct comparison against vehicle in LD (NANO-LD vs VEH-LD) confirmed these nanoceria-specific effects (Fig. 4). Together, these results indicate that nanoceria both quell inflammatory/apoptotic cascades and engage pro-survival networks that promote transcriptomic recovery.

Table 2

Summary of Differential Expression and Enriched Pathways Across Comparisons. For each comparison, the table reports the total number of significant differentially expressed genes (DEGs) with the count of up- (↑) and down-regulated (↓) genes, alongside the key significantly enriched Gene Ontology (GO) terms and KEGG pathways.

Comparison	Significant DEGs (Up/Down)	Key Enriched Pathways (GO/KEGG)
LD vs CTRL	920 (666 ↑ / 254 ↓)	↑ Inflammatory response, apoptosis, NF-κB, TNF signaling; ↓ phototransduction, metabolic genes
VEH vs CTRL	38 (17 ↑ / 21 ↓)	No significant pathways (minimal changes)
VEH-LD vs LD	75 (35 ↑ / 40 ↓)	Subtle ↑ wound response (e.g., <i>Rptn</i>); ↓ complement system (not significant)
NANO vs CTRL	306 (243 ↑ / 63 ↓)	↑ Antioxidant activity, mitochondrial metabolism; ↓ apoptosis (caspase activation)
NANO-LD vs LD	78 (36 ↑ / 42 ↓)	↑ Neurotrophin signaling, Notch, antioxidant pathways; ↓ NF-κB/TNF signaling, inflammasome
NANO-LD vs VEH-LD	34 (17 ↑ / 17 ↓)	↑ Synaptic plasticity, PI3K/Akt, oxidative phosphorylation; ↓ chemokines, cytokines, complement

2.5. Nanoceria uncover “non-canonical” axes: amino-acid/urea-cycle and insulin/glucose signaling

Beyond antioxidant and inflammatory pathways, nanoceria modulated amino-acid metabolism/urea-cycle genes (e.g., *Ass1*, *Cps1*, *Otc*), reversing their LD-associated repression and elevating expression toward or above baseline, consistent with enhanced nitrogen handling and anaplerotic support. In parallel, nanoceria increased insulin/IGF signaling components (*Insr*, *Irs1*) and the insulin-responsive glucose transporter *Slc2a4* (Glut4), suggesting improved glucose utilization and coupling to PI3K–Akt survival signaling in stressed retina. These axes were not appreciably engaged by vehicle and represent novel, testable mechanisms by which nanoceria may stabilize retinal bioenergetics (Supplementary Fig. S7; Supplementary Table S6).

2.6. qRT-PCR validates key signatures

Targeted qRT-PCR corroborated RNA-seq trends: *Irf6* rose after LD and decreased with nanoceria, whereas *Sod2* and *Nfe2l2* increased with LD and remained elevated under nanoceria, consistent with reinforced antioxidant defense. RNA-seq vs qPCR values showed strong concordance (e.g., Pearson’s $r \approx 0.90$ – 0.97 across genes; $p < 0.05$), supporting dataset robustness (Fig. 5).

3. Discussions

Cerium oxide nanoparticles (nanoceria) possess unique redox-switching properties, enabling them to catalytically and repeatedly scavenge reactive oxygen species (ROS), owing to their ability to cycle between Ce^{3+} and Ce^{4+} oxidation states^{10,11}. This regenerative antioxidant capacity has positioned

nanoceria as promising candidates for the treatment of oxidative stress-driven retinal disorders such as age-related macular degeneration (AMD), retinitis pigmentosa (RP), and diabetic retinopathy^{12,13}. In preclinical models of retinal degeneration, intravitreal administration of nanoceria consistently preserved retinal morphology and function. For example, in the *Vldlr*^{-/-} mouse model of AMD, a single intravitreal nanoceria injection suppressed aberrant neovascularization and reduced pro-inflammatory gene expression (e.g., VEGF, IL-6, IL-17), while restoring retinal thickness and electrophysiological responses. Similarly, topical application of nanoceria eye drops restored transcriptomic homeostasis and anatomical integrity in AMD rat models, suggesting that even non-invasive administration can reverse disease-associated gene expression profiles¹². These effects were accompanied by significant downregulation of stress kinases (e.g., JNK, p38) and inflammatory cytokines, underscoring nanoceria's multi-layered neuroprotective effects on the degenerating retina. Our current transcriptomic findings confirm and extend these observations: nanoceria pre-treatment not only suppressed classical inflammatory mediators such as TNF- α and IL-1 β , but also restored expression of neurotrophic and antioxidant genes, thus realigning the retinal transcriptome toward a protective, homeostatic state. Importantly, these effects go beyond passive antioxidant activity. By modulating upstream signaling networks—including MAPK, NF- κ B, and PI3K/Akt—nanoceria appear to actively influence cellular stress responses and shift the balance from degeneration toward survival¹³. Unlike conventional antioxidants such as vitamin C or E, which are stoichiometrically consumed and require frequent dosing, nanoceria operate catalytically and are retained in the retina for extended periods (up to 12 months), maintaining functional antioxidant activity without inducing significant toxicity^{10,14}. Collectively, these properties reinforce the therapeutic value of nanoceria as a next-generation, long-acting neuroprotective nanomedicine for retinal disease.

A principal mechanism by which nanoceria exert neuroprotection in the retina is through attenuation of oxidative stress and suppression of inflammatory signaling cascades. Retinal degenerative diseases—including age-related macular degeneration (AMD), retinitis pigmentosa (RP), and diabetic retinopathy—are driven by chronic oxidative damage and persistent low-grade inflammation that promote photoreceptor apoptosis and retinal pigment epithelium (RPE) dysfunction¹⁵. Nanoceria, via redox-active Ce³⁺/Ce⁴⁺ cycling, act as self-regenerating antioxidants capable of neutralizing superoxide, hydrogen peroxide, and peroxynitrite^{16,17}. Our transcriptomic analysis supports this: in light-damaged retinas, nanoceria significantly downregulated proinflammatory genes such as *Tnf*, *Il1b*, *Il6*, and *Ccl2*. At the signaling level, we observed decreased expression of Tlr4 and Myd88, consistent with inhibition of the Toll-like receptor (TLR)–mediated NF- κ B pathway. This pathway is a well-established trigger of inflammatory transcriptional programs and microglial activation in retinal degeneration. Supporting this, previous studies demonstrated that cerium oxide nanoparticles attenuate TLR4/NF- κ B activation in brain inflammation models, reducing cytokine production and microgliosis¹⁸. In retinal tissue specifically, Badia et al.¹² demonstrated that CeO₂-NPs reduce microglial activation and suppress TNF- α expression following oxidative stress. These anti-inflammatory effects appear selective and non-immunosuppressive. Instead of silencing broad immune responses, nanoceria specifically interrupt

pathological inflammatory cycles while preserving beneficial immune functions such as phagocytosis and matrix remodeling. For instance, in our dataset, *Timd4* and *Ctse*—genes involved in apoptotic cell clearance and lysosomal remodeling—were upregulated, indicating enhanced homeostatic responses. Nanoceria's antioxidant action further complements this immunomodulatory profile. By lowering ROS levels, nanoceria suppress upstream NF-κB activators such as oxidized mitochondrial DNA and lipid peroxidation products¹⁶. We also observed activation of Nrf2 signaling (*Nfe2l2*), a master regulator of redox balance. Nanoceria-treated retinas exhibited significant upregulation of canonical Nrf2 target genes: *Hmox1*, *Gclc*, *Nqo1*, and *Sod2*, aligning with prior evidence of Nrf2-mediated neuroprotection by cerium nanoparticles^{13,17}. This coordinated suppression of TLR4–MyD88–NF-κB and enhancement of Nrf2-driven antioxidant defense supports a dual mechanism by which nanoceria modulate inflammation and oxidative injury in the retina. Such combined action may be especially advantageous in retinal diseases where these pathways are interlinked in a self-amplifying loop. Our transcriptomic data reinforce this model. Chemokine genes *Ccl2*, *Ccl3*, and *Cxcl10*, which are known drivers of neuroinflammation in light-damaged retinas, were significantly downregulated by nanoceria. Notably, *Ccl2* is a key mediator of microglial recruitment and photoreceptor apoptosis¹⁹, while *Ccl3* and *Cxcl10* participate in chemokine-driven neurodegeneration²⁰. Proinflammatory cytokines *Il6* and *Tnf* followed expected dynamics: induced after photic injury and suppressed by nanoceria. These cytokines are well-characterized effectors of gliosis and retinal damage. The glial stress marker *Gfap*, upregulated during Müller cell activation, was also reduced by nanoceria, indicating mitigation of reactive gliosis¹⁹. Conversely, the neuroprotective factor *Fgf2* remained elevated in nanoceria-treated groups, in line with its role in photoreceptor survival²¹. Downregulation of *Aif1* (IBA-1), a microglial activation marker, further supports nanoceria's capacity to suppress innate immune activation³. These transcriptomic signatures strongly support the view that nanoceria modulate the oxidative stress–inflammation axis in a precise and therapeutically favorable manner, reinforcing their potential as neuroprotectants in retinal disease.

Nanoceria's neuroprotective activity extends beyond antioxidative effects and includes direct modulation of apoptosis, stress response, and pro-survival pathways. Multiple transcriptomic and qPCR studies have demonstrated that nanoceria treatment downregulates pro-apoptotic genes such as *Casp3*, *Bax*, and *Il1b*, while promoting expression of pro-survival factors including *Bcl2*, *Sirt1*, and neurotrophic genes like *Bdnf*, *Cntf*, and *Ngf*²². These changes correlate with delayed photoreceptor cell death and preservation of retinal architecture observed in histological analyses. In the present study, we observed significant suppression of *Casp3*, *Il1b*, *Tnf*, and *Nlrp3* following nanoceria treatment in the LD model, both in direct comparisons and pathway enrichment. These effects were accompanied by the activation of *Nrf2* (*Nfe2l2*) and its downstream targets *Hmox1*, *Gclc*, and *Nqo1*, key components of the antioxidant defense system. The role of Nrf2 in protecting against oxidative and proteotoxic stress in neurons and glia has been firmly established, and it represents a viable therapeutic target in AMD and Parkinson's disease²³. Importantly, nanoceria-mediated activation of Nrf2 has also been associated with improved mitochondrial quality control. In dopaminergic neurons, CeO₂ nanoparticles reduce mitochondrial

fragmentation by inhibiting DRP1 hyperactivation and peroxynitrite formation²². Consistently, our ultrastructural data showed preserved mitochondrial morphology in nanoceria-treated retinas, contrasting with the disrupted, swollen mitochondria observed in untreated LD eyes. Transcriptomic analysis further revealed upregulation of stress-responsive transcription factors *Atf3* and *Sirt1*, both associated with cellular recovery following oxidative and metabolic insults. *Sirt1*, in particular, is known to support mitochondrial biogenesis, energy homeostasis, and antioxidant defense by interacting with FoxO and PGC-1 α pathways²³. The observed increase in *Sirt1* expression in treated animals may synergize with *Nrf2* to promote neuroprotection, especially in the high-energy-demand environment of photoreceptors. An unexpected finding was the upregulation of regeneration-associated transcription factors such as *Ascl1*, *Sox2*, and *Notch1* in nanoceria-treated retinas. These factors are typically quiescent in the adult mammalian retina but are central to the Müller glia reprogramming network in regenerative species like zebrafish²⁴. Their re-expression in this context suggests that nanoceria may induce a transcriptional “priming” effect, rendering the retina more responsive to potential regenerative cues. Although histological analysis did not reveal active regeneration or Müller cell proliferation, the transcriptional activation of these genes is noteworthy and may offer a window of opportunity for synergistic therapeutic interventions (e.g., gene therapy or trophic factor delivery). Taken together, our results underscore nanoceria’s multifaceted action on survival and stress-response mechanisms. This includes suppression of inflammation and apoptosis, enhancement of antioxidant and mitochondrial defense via *Nrf2* and *Sirt1*, and activation of latent regenerative programs. These concerted transcriptomic changes shift the degenerating retina from a trajectory of degeneration toward stabilization and potentially recovery.

One of the most novel findings in our current study is the transcriptomic evidence that nanoceria significantly modulate retinal metabolic pathways—particularly amino acid catabolism and insulin/glucose signaling—which are rarely addressed in nanomedicine-focused neuroprotection studies²⁵. We observed that nanoceria restored the expression of enzyme encoding genes such as *Ass1* (argininosuccinate synthase 1) and *Cps1* (carbamoyl-phosphate synthetase 1), which are central to the urea cycle and nitrogen handling. The presence and modulation of these enzymes in retinal tissue, especially their stress-induced downregulation and restoration by nanoceria, suggest that metabolic reprogramming is a key component of retinal degeneration—and a modifiable one. In untreated light-damaged retinas, these genes were suppressed, indicating metabolic dysfunction. This mirrors findings in other neurodegenerative models where ammonia accumulation and nitrogen imbalance promote excitotoxicity and glial dysfunction²⁶. The restoration of ASS1 and CPS1 by nanoceria aligns with their known roles in ammonia detoxification and arginine availability, both of which are crucial for nitric oxide balance and polyamine synthesis—two pathways linked to synaptic function and redox homeostasis²⁷. Additionally, nanoceria markedly upregulated key insulin signaling components in the retina, including *InsR*, *Irs1*, and *Slc2A4* (GLUT4). This suggests that nanoceria may counteract retinal insulin resistance, a phenomenon increasingly recognized in both AMD and diabetic retinopathy²⁸. Insulin signaling not only governs glucose uptake but also activates the PI3K/Akt pathway, which promotes neuronal survival and

inhibits apoptosis. In our model, transcriptional recovery of this axis indicates that nanoceria may restore metabolic competence to injured photoreceptors and glia—safeguarding ATP production under oxidative stress conditions. This insulin-sensitizing effect has been reported in peripheral metabolic and neurodegenerative conditions, such as Alzheimer’s disease²⁹ but is novel in the context of retinal degeneration. Our data now position nanoceria at the intersection of redox regulation and nutrient signaling—a dual role that may explain their broad neuroprotective activity. Furthermore, by lowering oxidative burden, nanoceria likely prevent secondary metabolic collapse and preserve mitochondrial function, as evidenced by upregulation of mitochondrial enzymes and the improved ultrastructure observed via electron microscopy³⁰. Finally, repression of inflammatory mediators such as *Tlr4*, *Myd88*, and NF-κB targets further supports coordinated suppression of the inflammation–metabolic stress axis. It is well established that TLR4/NF-κB signaling not only drives proinflammatory cytokine production but also impairs insulin sensitivity and mitochondrial bioenergetics^{31,32}. In summary, our findings add a new dimension to nanoceria’s mechanism of action by identifying metabolic reprogramming—particularly involving the urea cycle and insulin signaling—as underexplored but central components of their therapeutic profile. This suggests that future neuroprotective strategies, especially for metabolically demanding tissues like the retina, could benefit from co-targeting these metabolic pathways alongside classical antioxidant and anti-inflammatory axes.

One of the most compelling emerging mechanisms by which nanoceria exert neuroprotective effects is their ability to stabilize autophagy and preserve mitochondrial function—two closely linked processes essential for retinal health. Chronic oxidative stress, a hallmark of retinal degenerative diseases such as AMD and RP, disrupts autophagic flux and promotes mitochondrial dysfunction, leading to bioenergetic collapse and cell death, particularly in metabolically demanding tissues like the retina and RPE³³. Recent studies have highlighted nanoceria’s role in preserving autophagic homeostasis. For example, in a light-damage-induced retinal degeneration model, intravitreal nanoceria treatment prevented abnormal accumulation of LC3B-II and p62 proteins in RPE cells, indicative of restored autophagic flux. Notably, nanoceria blocked aberrant nuclear localization of LC3B—a feature associated with impaired autophagosome maturation and epithelial-mesenchymal transition (EMT) in degenerating RPE¹⁴. By maintaining normal autophagy, nanoceria preserved RPE integrity and prevented cell death. Complementing these molecular findings, ultrastructural evidence from previous studies demonstrates that nanoceria-treated retinas maintain mitochondrial integrity after injury³⁴. In our rat model, TEM analysis revealed that mitochondria in nanoceria-injected eyes retained cristae structure and showed fewer signs of swelling or fragmentation compared to untreated LD eyes, which exhibited extensive mitochondrial damage. These observations align with prior work in neurodegenerative models where nanoceria localized to mitochondria and prevented excessive fission by inhibiting DRP1 hyperactivation, a key step in the mitochondrial apoptotic cascade³³. Mechanistically, nanoceria appear to exert these effects through multiple routes:

1. Direct ROS scavenging, reducing mitochondrial oxidative burden;

2. Suppression of inflammation that can secondarily impair mitophagy;
3. Stabilization of Nrf2 signaling, which indirectly supports mitochondrial biogenesis and autophagy-related gene expression (e.g., PINK1, Parkin) ³⁵.

Indeed, transcriptomic data from our model show that genes involved in mitochondrial dynamics—Opa1, Mfn1, and Tomm20—were better preserved in nanoceria-treated retinas, suggesting maintenance of mitochondrial fusion and protein import systems. This preservation of mitochondrial and lysosomal health is significant. In AMD, defective autophagy and impaired mitophagy contribute to RPE degeneration, lipofuscin accumulation, and drusen formation. Therapies that restore autophagic flux and mitochondrial clearance—such as rapamycin or metformin—have shown benefit in animal models, but often with systemic side effects. Nanoceria, by contrast, provide a local, long-acting alternative that appears to accomplish similar outcomes through redox modulation and transcriptional reprogramming without systemic toxicity. In sum, the impact of nanoceria on autophagy and mitochondrial maintenance adds a crucial dimension to their neuroprotective repertoire. By targeting fundamental organelle-level processes that govern cell survival, nanoceria go beyond antioxidant action and engage with the core bioenergetic and proteostatic machinery of retinal cells. This capacity to stabilize intracellular quality control systems, particularly under stress, likely underlies the long-term structural and functional protection observed in treated retinas.

Taken together, the transcriptomic data across all comparisons reveal that nanoceria exerts a profound and multi-dimensional modulatory effect on the degenerating retina. In the context of light-induced injury, nanoceria sharply attenuates the expression of inflammatory mediators (e.g., *Tnf*, *Il1b*, *Il6*, *Ccl2*) while upregulating key neuroprotective and metabolic genes—including *Bdnf*, *Cntf*, *Sirt1*, and *Nfe2l2*. These transcriptional changes are reflected in the enrichment of biological processes related to oxidative stress resistance, mitochondrial function, neuronal survival, and synaptic maintenance. Interestingly, nanoceria also induced cytoprotective and metabolic programs in healthy (uninjured) retina, including the upregulation of antioxidant pathways and mitochondrial enzymes. This finding suggests a preconditioning effect, whereby nanoceria enhances the retina's baseline resilience even before damage occurs. Most notably, beyond expected antioxidant and anti-inflammatory effects, our data uncover previously unreported nanoceria-regulated pathways. These include:

- Metabolic activation of amino acid biosynthesis and the urea cycle (*Cps1*, *Ass1*, *Otc*);
- Upregulation of insulin/IGF signaling components (*Insr*, *Irs1*, *Slc2a4*);
- Suppression of innate immune sensors such as *Tlr4* and *Myd88*, dampening NF-κB signaling;
- Induction of developmental transcription factors (*Ascl1*, *Sox2*, *Notch1*, *Wnt2b*), often quiescent in the adult retina, and linked to regenerative competence.

These observations, summarized in Table 2, suggest that nanoceria not only blunts degeneration-associated cascades, but reprograms the retinal transcriptome toward a reparative state—though not completely reversing damage, it selectively reinforces protective and regenerative programs.

Mechanistically, the activation of insulin signaling could enhance photoreceptor survival through PI3K/Akt/mTOR pathways, while suppression of TLR4–MyD88–NF-κB signaling reduces glial activation and cytokine-mediated secondary damage. Simultaneously, the partial reactivation of regenerative factors raises the possibility that nanoceria fosters a more permissive, plastic environment in the adult mammalian retina, echoing features of regenerative species. These insights expand nanoceria's profile from a ROS-scavenger to a pleiotropic modulator of retinal homeostasis—acting on metabolism, inflammation, and repair. Importantly, several of these pathways (e.g., the urea cycle, insulin signaling, Wnt/Notch activation) have not been previously linked to nanoceria in vivo, underscoring the novelty and translational relevance of our findings. In light of these observations, nanoceria emerges as a candidate for multimodal therapeutic strategies in complex retinal diseases such as AMD and RP, where oxidative stress, immune activation, and metabolic dysregulation converge. Combining nanoceria with metabolic cofactors or targeted modulators of regenerative signaling (e.g., Wnt/Notch agonists) may further amplify its protective effects.

4. Conclusions and Future Directions

Our transcriptomic analysis provides compelling evidence that cerium oxide nanoparticles (nanoceria) elicit a multifaceted neuroprotective response in the retina under both homeostatic and degenerative conditions. In a rat model of light-induced retinal degeneration, nanoceria administration not only attenuated classical markers of oxidative stress and inflammation but also preserved metabolic signaling pathways, restored components of cellular homeostasis, and induced elements of developmental and regenerative transcriptional programs. At the molecular level, nanoceria-treated retinas exhibited reduced expression of genes encoding pro-inflammatory mediators such as TNF-α, IL-6, and CCL2, alongside downregulation of the TLR4–MyD88–NF-κB signaling axis, which is often implicated in retinal and neuroinflammatory pathologies. Concomitantly, we observed the activation of survival-promoting pathways such as PI3K/Akt, neurotrophin signaling, and antioxidant networks under control of *Nfe2l2* (*Nrf2*). Unexpectedly, nanoceria also restored the expression of metabolic regulator genes like *Insr* and *Glut4*, and modulated amino acid and nitrogen metabolism (e.g., ASS1 and CPS1), pointing to novel roles in sustaining retinal bioenergetics and limiting toxic metabolic byproducts. Furthermore, nanoceria triggered the expression of genes typically associated with retinal regeneration in lower vertebrates (e.g., *Notch1*, *Sox2*, *Ascl1*), suggesting that even in the adult mammalian retina, nanoceria may partially reawaken latent regenerative programs. While true neurogenesis was not observed histologically, the transcriptional reprogramming indicates a shift toward a reparative retinal environment—a finding with major implications for regenerative therapeutics. From a translational perspective, these results reinforce the broad-spectrum potential of nanoceria as a next-generation therapy for retinal degenerative diseases such as AMD and RP. Their intrinsic regenerative redox cycling enables persistent antioxidant activity, and recent advances in delivery platforms (e.g., eye drops, sustained-release hydrogels, intranasal administration) support their clinical feasibility. Importantly, no overt toxicity has been observed in long-term in vivo models, and multiple independent studies across neurodegenerative systems (retina, brain) confirm their efficacy and safety. In conclusion, nanoceria

represent a promising disease-modifying agent capable of intervening at multiple levels of retinal degeneration—quenching oxidative and inflammatory cascades, preserving cellular metabolism, and possibly priming repair pathways. These insights expand our understanding of nanoceria’s mode of action and lay the groundwork for future preclinical and clinical studies aimed at establishing nanoceria-based interventions as a therapeutic reality in ophthalmology and beyond.

5. Methods

5.1. Animal model and ethical approval

Adult albino Sprague–Dawley rats ($\approx$ 2–3 months old, males) were used for all experiments. Rats were born and raised under dim cyclic lighting (12 h light/12 h dark) at constant temperature ($\sim$ 22°C) and humidity, with diurnal light $\approx$ 5 lux. Food (standard pellet diet) and water were provided ad libitum. Animals were randomly assigned to experimental groups (n = 6 per group) including untreated controls, light-damaged (LD) only, and nanoceria-treated + LD groups (additional vehicle-injected controls were included for injection procedures). All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the European Directive 2010/63/EU and were approved by the Italian Ministry of Health (authorization no. 763/2020-PR). The authors complied with the ARRIVE guidelines. All efforts were made to minimize animal suffering and to reduce the number of animals used in accordance with the 3Rs principle. To better clarify the experimental design, the seven experimental groups are summarized in Table 3.

Table 3
Experimental groups and treatment conditions. Summary of the six groups (CTRL, LD, VEH, NANO, VEH-LD, NANO-LD; n = 6 each) and interventions (light exposure, intravitreal saline, intravitreal nanoceria).

Experimental Groups	Treatment	N
CTRL	No treatment	6
LD	1000 lux exposure	6
VEH	Intravitreal injection of saline	6
NANO	Intravitreal injection of nanoceria	6
VEH-LD	Saline injection + 1000 lux exposure	6
NANO-LD	Nanoceria injection + 1000 lux exposure	6

Then, to induce retinal degeneration and recapitulate the features of AMD, albino rats were exposed to high intensity light for 24 h. The detailed experimental procedures were already reported in previous papers^{3,4,8,9,14,36}.

5.2. Nanoceria preparation and intravitreal injection

Cerium oxide nanoparticles (nanoceria, CeO₂-NPs) were synthesized via a hydrothermal method as described previously³⁷. The resulting nanoceria colloid was water-dispersible and consisted of crystalline particles ~ 2–10 nm in diameter as observed by transmission electron microscopy (TEM). Dynamic light scattering (DLS) confirmed a small hydrodynamic size (on the order of ~ 10 nm), and the ζ-potential of the nanoparticles at 1 mM in saline was approximately + 10 ± 3 mV, indicating modest colloidal stability. Each batch of nanoceria was tested for endotoxin contamination using a Limulus amoebocyte lysate (LAL) assay, and endotoxin levels were below the detection limit (< 0.1 EU/mL). For in vivo administration, nanoceria were sterile-filtered (0.22 μm) and prepared at 1 mM in sterile 0.9% NaCl. Under anesthesia (intraperitoneal ketamine 100 mg/kg + xylazine 10 mg/kg), rats received a bilateral intravitreal injection of 2 μL of the nanoceria suspension (1 mM) in each eye using a Hamilton microsyringe with a fine gauge needle. In the vehicle control group, 2 μL of sterile saline was similarly injected. Injections were performed 3 days prior to light exposure (i.e., on day – 3 relative to LD) to allow nanoceria distribution in the retina before damage. All intravitreal injections were done under aseptic conditions using a surgical microscope; afterward, topical antibiotic ointment was applied to the eyes. No signs of infection or overt ocular damage were observed following the procedure. Rats were closely monitored during recovery from anesthesia.

5.3. RNA extraction and quality control

Seven days post-light exposure (or at corresponding time points for control animals), rats were euthanized by CO₂ inhalation and eyes were immediately enucleated. Retinas were rapidly dissected from each eye on ice. Total RNA was extracted from retinal tissue using TRIzol™ Reagent (Invitrogen, ThermoFisher Scientific) according to the manufacturer's instructions. RNA quantification was performed using the Qubit RNA HS Assay Kit (Thermo Fisher Scientific), and the RNA integrity was assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies); only high-quality RNA samples with RNA Integrity Number (RIN) ≥ 7.0 were used for library preparation.

5.4. Library preparation and sequencing

For each sample, 1 μg of total RNA was processed for poly(A) + mRNA enrichment. Messenger RNA was isolated using oligo(dT) magnetic beads and converted to strand-specific cDNA. Strand-specific sequencing libraries were prepared using the Watchmaker RNA Library Prep Kit (Twist Bioscience) following the manufacturer's protocol. Adapter ligation, PCR amplification (with unique dual indexing for multiplexing), and library purification were performed as outlined in the Watchmaker workflow. Post-amplification, library quality and insert size distributions were assessed using a Fragment analyzer, confirming fragment sizes of 250–350 bp. Equimolar pools of indexed libraries were sequenced on an Illumina NovaSeq 6000 system to generate 150 bp paired-end reads, with each retina library sequenced to a mean depth of ~ 80 million reads. Raw sequencing data (FASTQ files) underwent quality control checks, including evaluation of base quality scores, GC content, and adapter contamination; all samples met predefined thresholds for downstream transcriptomic analysis.

5.5. RNA-seq data analysis

Primary analysis of RNA-seq data was carried out using a composite bioinformatics pipeline combining a canonical open-source workflow with CLC Genomics Workbench (v25.0.0) analyses. In the canonical pipeline, raw reads were first processed using fastp (v0.20) for adapter trimming and quality filtering³⁸. Fastp performed per-read quality pruning and removed Illumina adapter sequences, yielding high-quality clean reads for each sample. Clean reads were then aligned to the *Rattus norvegicus* reference genome (e.g., Rnor_6.0) using the STAR aligner (v2.7.11) with default parameters for two-pass spliced read mapping³⁹. Mapping quality was high, with the majority of reads uniquely aligned to exonic regions of the genome. Gene-level read counts were computed using featureCounts (v2.0) from the Subread package, summarizing aligned reads per gene based on Ensembl gene annotation⁴⁰. The resulting raw count matrix was imported into R (v4.1) for downstream analysis. DESeq2 (v1.48.0) was used to normalize gene counts and identify differentially expressed (DE) genes between experimental groups⁴¹. The DESeq2 model accounted for biological replicate variability, and shrinkage estimators were applied to dispersions and fold changes. Genes with a false discovery rate (FDR)-adjusted p-value < 0.05 were considered significantly differentially expressed. No fold-change cutoff was applied; all genes passing the FDR threshold were retained. Unsupervised sample clustering via principal component analysis (PCA) was performed to evaluate sample relationships and detect potential outliers. PCA results were visualized with violin plots, showing the distribution and density of sample loadings across the first three principal components (PC1–PC3), thereby highlighting transcriptional heterogeneity and treatment-specific clustering. Results from the canonical pipeline were then compared with those obtained via CLC Genomics Workbench, improving the reliability and consistency of DE findings. DE gene profiles were visualized with volcano plots and heatmaps showing the top regulated genes. To interpret transcriptomic alterations functionally, up- and down-regulated gene sets were analyzed for gene ontology (GO) and KEGG pathway enrichment using the clusterProfiler R package⁴². Enrichment testing used an FDR cutoff of 0.05. Significant GO terms (Biological Process, Molecular Function, Cellular Component) and KEGG pathways were visualized using dot plots and enrichment maps, providing insights into nanoceria-modulated biological processes in retinal degeneration. All data visualizations (PCA, heatmaps, volcano plots, enrichment plots) were generated in R using ggplot2⁴³ or clusterProfiler-native plotting functions.

5.6. Real-Time PCR Validation of Differentially Expressed Genes

To validate the RNA-seq results, we performed quantitative real-time PCR (qRT-PCR) analysis on a selection of differentially expressed genes (DEGs) identified across experimental groups. Specific primer pairs were designed for key upregulated and downregulated genes, including *Nfe2l2* (*Nrf2*), *IL6*, and *Sod2*. Total RNA was extracted from retinal tissue samples using TRIzol Reagent, followed by cDNA synthesis. qRT-PCR reactions were carried out using CFX96 Real Time System (Bio-Rad) and IQ SYBR Green Supermix (Bio-Rad) according to the manufacturer instructions. Each reaction was performed in technical triplicate. Relative expression levels were normalized to the housekeeping gene for actine, and the $\Delta\Delta C_t$ method was used to quantify fold changes.

5.7. Statistical analysis

For non-transcriptomic measurements (e.g., nanoparticle characterization, histological quantification), data are presented as mean $\pm$ standard error of the mean (SEM), unless otherwise specified. Statistical analyses were conducted using GraphPad Prism⁴⁴ and R⁴⁵. Group comparisons were performed using appropriate parametric tests. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied. For pairwise group comparisons, unpaired two-tailed Student's t-tests were used. A significance threshold of $p < 0.05$ was employed for all statistical tests unless otherwise stated. Statistical significance for differential gene expression in RNA-seq data was assessed as described in Section 2.5, based on adjusted p-values calculated using the Benjamini-Hochberg false discovery rate (FDR) method.

Declarations

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Author contributions

D.Z., R.M. and L.D. conceptualized and designed the study. M.P., V.F., M.F. and C.R. contributed to data acquisition. L.D., I.B. and D.Z. analyzed the data. L.D. and D.Z. drafted the manuscript. R.M., R.D., M.C., L.P. critically reviewed and revised the manuscript. R.M., R.D., L.P. obtained the funds. D.Z., R.M., R.D. and L.P. supervised the study.

Additional Information

Data availability: all relevant data generated or analyzed during this study are available from the corresponding author upon reasonable request. The raw RNA-seq data (FASTQ files) and processed count matrices have been deposited in the NCBI Gene Expression Omnibus (GEO) under the accession number GSE306375. Additional datasets supporting the findings of this study—including nanoparticle characterization data and histological images—are provided in the Supplementary Information or are available upon request from the authors.

Competing interests: the authors declare no competing interests.

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Figures

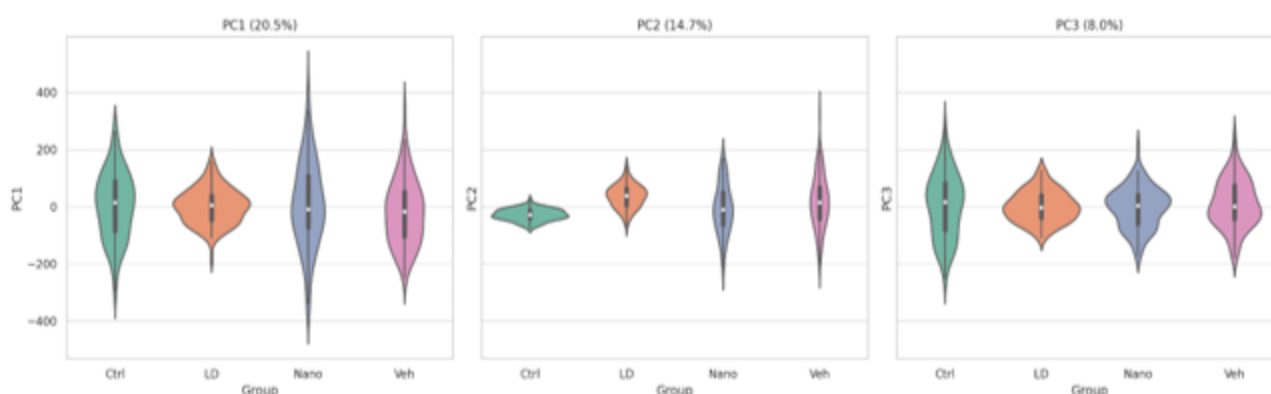


Figure 1

Global sample structure by PCA. Principal component analysis of variance-stabilized RNA-seq counts shows clear separation among experimental groups. Light-damaged samples diverge from controls along the primary components, while nanoceria-treated samples (with or without light damage) shift away from their matched controls, consistent with a nanoceria-modulated transcriptional program. Biological replicates (n = 6 per group) cluster tightly. Statistical significance assessed by one-way ANOVA (two-tailed) with Tukey’s post-hoc test, $\alpha=0.05$. Group size n=6 animals per group. Data are mean $\pm$ SEM. $p < 0.05$.

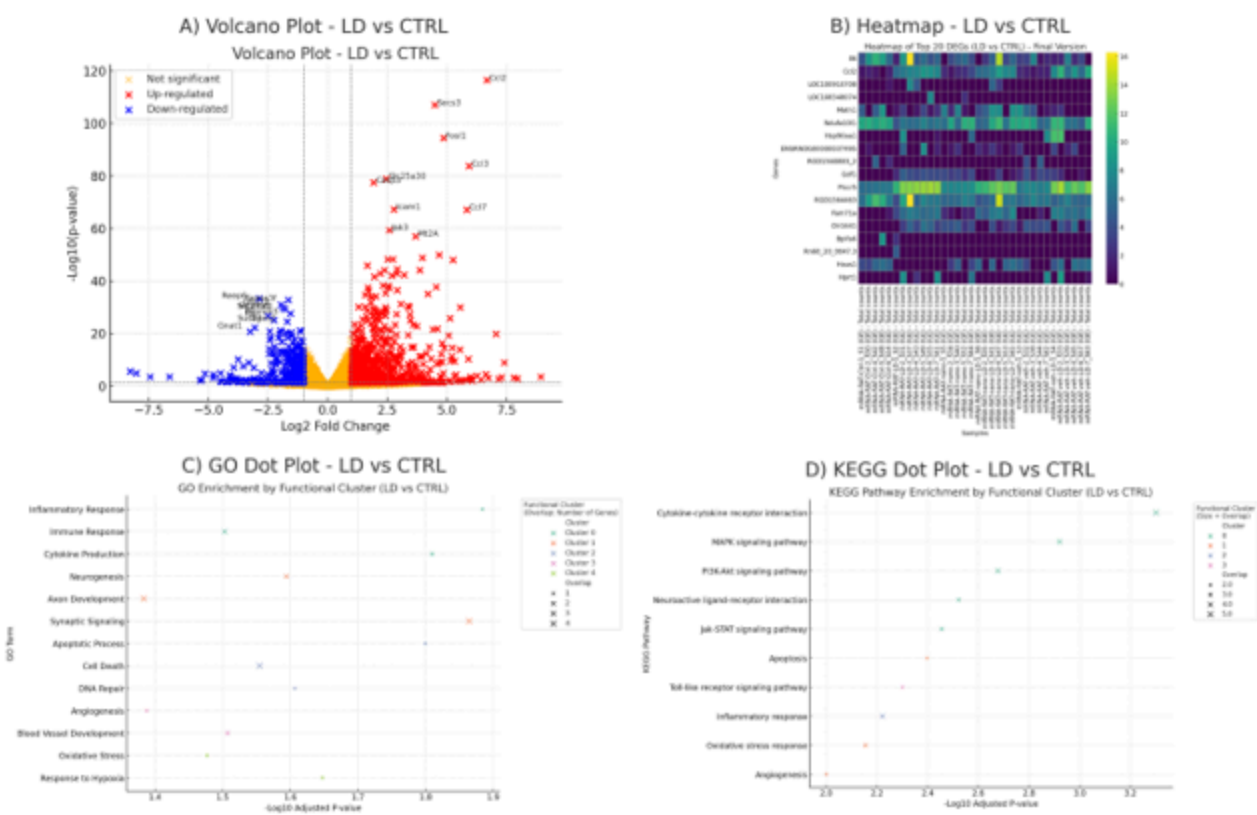


Figure 2

Light damage (LD) activates inflammatory/apoptotic programs and suppresses phototransduction. (A) Volcano plot for LD vs CTRL (FDR < 0.05) highlighting upregulation of cytokines/chemokines (e.g., *Tnf*, *Il6*, *Il1b*, *Ccl2/Ccl3/Ccl4*) and gliosis markers, with strong downregulation of photoreceptor/visual-cycle transcripts (e.g., *Rho*, *Pde6a/b*, *Gnat1*, *Cnga1*; *Crx*, *Nrl*, *Nr2e3*). (B) Heatmap of top DEGs illustrates clear CTRL vs LD segregation. (C–D) Summary enrichment (GO/KEGG) indicates $\uparrow$ inflammatory response / TNF–NF- κ B / apoptosis and $\downarrow$ phototransduction / retinol metabolism; full enrichment in Fig. S1. Differential expression determined with DESeq2, using Wald test and Benjamini–Hochberg correction. Significance defined at adjusted p (FDR) < 0.05. Sample size: n=6 biological replicates per group.

Pathway information was obtained from the KEGG database and is reproduced with permission from Kanehisa Laboratories (© Kanehisa Laboratories).

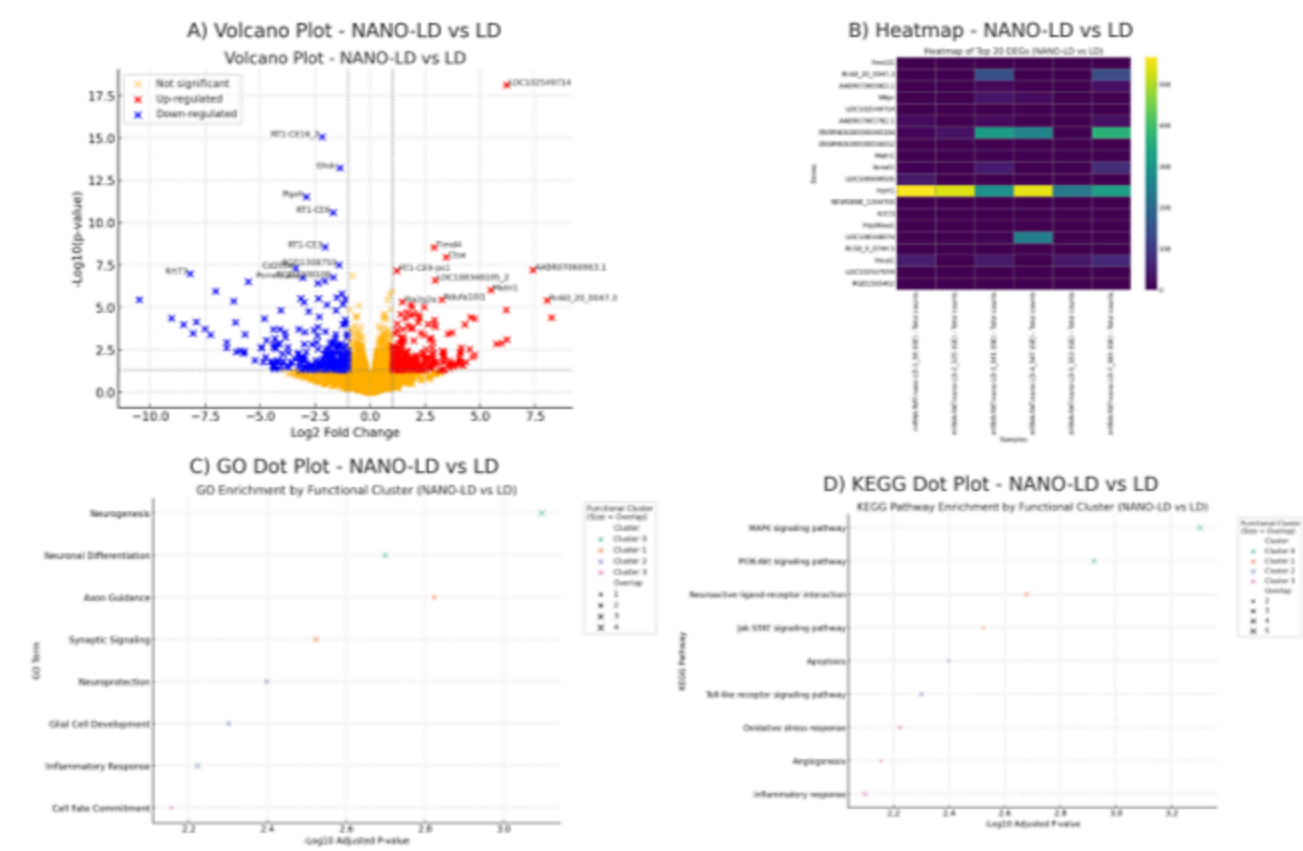


Figure 3

In injured retina, nanoceria oppose LD-induced signatures and enhance pro-survival networks. (A) Volcano for NANO-LD vs LD (FDR < 0.05) shows downregulation of inflammatory mediators (*Tnf*, *Il6*, *Il1b*, *Ccl2*, *Nfkbia*) and gliosis markers (*Gfap*, *Aif1*) with preservation/recovery of photoreceptor transcripts (*Rho*, *Pde6b*). (B) Heatmap of representative DEGs. (C–D) Pathway summaries indicate suppression of TNF/NF-κB/IL-17 and activation of PI3K–Akt, JAK–STAT, neurotrophin signaling, alongside strengthened oxidative phosphorylation and mitochondrial organization (full enrichment: Fig. S6). Differential expression determined with DESeq2, using Wald test and Benjamini–Hochberg correction. Significance defined at adjusted p (FDR) < 0.05. Sample size: n=6 biological replicates per group. Pathway information was obtained from the KEGG database and is reproduced with permission from Kanehisa Laboratories (© Kanehisa Laboratories).

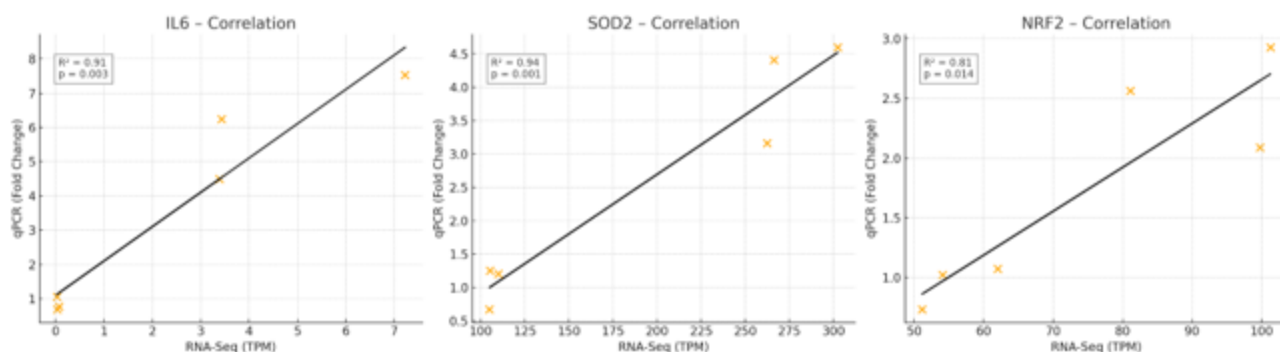


Figure 5

Correlation between RNA-Seq and qRT-PCR expression for *Il6*, *Sod2*, and *Nrf2*. Scatter plots show a strong concordance between transcriptomic and qPCR data across treatment groups. Expression values from RNA-Seq (TPM) are plotted against corresponding qPCR fold changes. All genes demonstrated robust and statistically significant linear correlations ($R^2 > 0.80$; $p < 0.05$), confirming the consistency of differential expression profiles between the two methodologies. $n=6$ animals per group, reactions in technical triplicate.

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