

Review

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Posted Date: 2 March 2026

doi: 10.20944/preprints202602.2055.v1

Keywords: regeneration, cell ablation, disease models, nitroreductase



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Review

The NTR/Prodrug Revolution: Tools for Controlling Cell Loss and Regeneration

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Abstract

Here, we review the history, advancements, and broad utility of the NTR/prodrug system, and suggest future strategies for developing versatile ablation models. As a chemogenetic tool, the nitroreductase (NTR)/prodrug system enables precise spatiotemporal control over cell ablation. The technology leverages bacterial nitroreductase enzymes (e.g., *nfsB*) to convert inert prodrugs into cytotoxic agents, thereby allowing researchers to induce targeted cell death. Following its landmark application in zebrafish with metronidazole (MTZ) in 2007, the system's utility has expanded to other essential model organisms, including *Drosophila*, *Nematostella*, *Xenopus*, medaka, and rodents, facilitating detailed studies of tissue damage and regeneration. This review highlights how the NTR system has been deployed to model a spectrum of human diseases, including Parkinson's disease, retinal degeneration, demyelinating disorders, and kidney disease. These models provide valuable platforms to study pathogenesis *in vivo*. Furthermore, the precise and controllable nature of NTR ablation makes it an ideal tool for high-throughput chemical and genetic screens aimed at discovering pro-regenerative and protective compounds. The development of NTR2.0, an enzyme variant with over 100-fold greater activity, along with more potent prodrugs such as ronidazole (RNZ), has dramatically broadened experimental possibilities. These improvements permit chronic ablation and long-term disease modeling at well-tolerated drug concentrations. Here we present some key considerations including transgenic design for optimal cell-type specificity, calibrating expression levels for desired ablation kinetics, and suitable controls to allow interpretation. These best practices will allow the researcher to develop a precise, reproducible, and versatile platform for either modeling human disease or dissecting regenerative mechanisms.

Introduction

Why regeneration?

We often regard ourselves as having limited regenerative capacity, yet numerous human tissues exhibit substantial renewal: the liver restores lost mass, the skin undergoes continuous stem cell-driven turnover, skeletal muscle repairs through satellite cells, bone heals via remodeling, blood is replenished by hematopoietic stem cells, the intestinal epithelium renews within days, and the endometrium regenerates cyclically. However, the regenerative capacity of human tissues is modest when contrasted with that of many other species, in which entire organs or appendages can be restored following injury [1,2]. This capacity is profound in many invertebrates; for instance, hydra and planarians can regenerate complete organisms from minute fragments [3].

Understanding these enviable examples provides a framework for uncovering the cellular and molecular mechanisms that organisms employ to repair or replace damaged tissues, with the ultimate goal of developing new strategies to combat human injuries, degenerative diseases, and age-related decline [4,5]. An essential requirement for this research is the ability to induce controlled, reproducible injury to study the subsequent repair processes.

The laboratory mouse (*Mus musculus*) is a critical vertebrate model for human biology and has been essential for developing inducible, cell-type-specific genetic tools in mammals. However, its intrinsic regenerative capacity is largely confined to a neonatal period, with abilities such as heart and digit tip regeneration declining sharply in adulthood [6]. The spiny mouse (*Acomys*) exhibits robust adult regeneration of skin, muscle, and ear tissue [7], but as an emerging model, it lacks the genetic toolkit for the systematic dissection of regenerative mechanisms.

For developing precise ablation tools, a genetically tractable and cost-effective vertebrate with robust adult regeneration is desirable. The zebrafish (*Danio rerio*) is uniquely suited for this role. Its external development, transparency, and high fecundity facilitate direct *in vivo* visualization and high-throughput screening [8]. Critically, zebrafish exhibit widespread regenerative abilities in adulthood, fully restoring complex organs including the heart [9,10], retina [11,12], spinal cord [13,14], and fins [15,16]. These attributes make zebrafish a practical system for dissecting the mechanisms of vertebrate regeneration. [17]

Methods of ablation

Researchers study regeneration by damaging tissue in different ways. The regenerative process that follows depends heavily on how the injury was created. The main types of injury model used in zebrafish can be classed as follows:

1) Physical Injury: Direct tissue damage via surgical intervention that have been used successfully to study adult regeneration, including fin amputation, [18] ventricular resection, [9] brain lesioning, [19] scale removal [20,21] and transection of both tendons [22] and ligaments. [23] Severing of the spinal cord predictably leads to paralysis in zebrafish, but as testament to their regenerative capacity, full mobility is returned by 8 weeks. [13,24,25]

Several non-surgical physical methods can be used to induce tissue damage in animal models, with their suitability largely determined by the subject's size and developmental stage. In adult models, approaches include cryoinjury to simulate myocardial infarction [26,27], intense light exposure to trigger photoreceptor degeneration in retinal regeneration studies [28], and acoustic trauma to model inner-ear damage and hearing loss recovery [29,30]. The acoustic method has also been applied to larval zebrafish to target lateral line hair cells [31]. Larval and embryonic zebrafish, owing to their small size and optical transparency, permit highly precise laser ablation of single neurons [32-34] or even individual cardiomyocytes [35]. Additionally, thermal injury has been employed in larvae to model burns and skin regeneration, revealing rapid inflammatory cell recruitment [36] and keratinocyte migration, but impaired sensory axon regeneration compared to mechanical injury [37].

2) Cell-Specific Toxins: While physical injury has provided key insights into regenerative biology, these approaches are often constrained by limited precision and cell-type specificity. Pharmacological methods offer a complementary strategy, using small molecules to induce targeted, dose-controlled damage. For instance, selective toxins are used to ablate specific cell populations:

- Aminoglycoside antibiotics ablate sensory hair cells [38-40].
- Streptozotocin (STZ) eliminates pancreatic β -cells [41]
- Ouabain targets retinal neurons [42,43].
- Neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) kill dopaminergic neurons, modeling aspects of Parkinson's disease [44].

However, these drugs are also hampered by a lack of true cellular specificity, often producing significant off-target effects that complicate experimental interpretation. Agents such as MPTP have the additional caveat of requiring strict handling procedures due to their high toxicity. To achieve higher precision and reduce off-target effects as well as expand the kinds of cell that can be ablated, the field has increasingly turned to genetic strategies. These methods allow damage to be targeted with exquisite specificity to predefined cell types.

3) Optogenetic cell ablation: Due to the transparent nature of young zebrafish, this model system is very amenable to optogenetic techniques that allow for precise, non-invasive control

of cellular signaling, neuronal circuit activity, and targeted cell ablation (see reviews [45,46]. KillerRed is a genetically encoded photosensitizer that, when illuminated with green or yellow light, produces reactive oxygen species (ROS) to kill nearby cells. [47,48] By driving its expression with cell-specific promoters, researchers can eliminate targeted cell types in living embryos or larvae with precise spatiotemporal control. [47-49] A key advantage of this technique is the speed of ablation, as transgene-expressing cells can be eliminated within hours of light exposure. This approach is particularly useful for modeling diseases in which ROS-mediated cell death plays a central role, such as neurodegeneration, [50] and cardiomyopathies, [51] but has not been applied in conventional regeneration studies.

4) Chemogenetic, cell-specific ablation: Chemogenetic ablation strategies use a transgene to drive cell-specific expression of an exogenous protein, rendering target cells susceptible to a specific chemical agent. Four such systems established in zebrafish are:

i) Human Diphtheria Toxin Receptor and Diphtheria toxin: The human pathogen *Corynebacterium diphtheriae* produces diphtheria toxin (DT). [52] This toxin binds to a specific human cell surface receptor called the diphtheria toxin receptor (hDTR) to enter and kill cells. [53] Most animals are resistant to DT because the toxin does not bind their endogenous receptors. A powerful experimental technique involves genetically engineering model organism to express the hDTR in a specific cell type. [54] When diphtheria toxin is injected into these animals, it only enters and ablates the cells expressing the hDTR, leaving all other cells unharmed.

Although widely used in mouse studies, its application in zebrafish is less common. [55,56] An exciting study by Jimenez et al. (2021) generated a transgenic zebrafish line in which the human diphtheria toxin receptor (hDTR) was expressed under the control of the hair-cell specific *myo6b* promoter. Upon diphtheria toxin administration, hair cells in the utricles, saccules, and lateral line were ablated in a dose-dependent manner in both larvae and adult zebrafish. Of note, complete regeneration of these sensory hair cells occurred within days, underscoring the value of this model for investigating targeted cell ablation and the mechanisms of hair cell regeneration across developmental stages. [55] Some studies have used transgenics to express subunit A of diphtheria toxin (DTA) in target cells, [57,58] but constitutive DTA expression causes cell loss without temporal control, precluding regeneration studies. Others have incorporated Cre/lox inducible systems to temporally control DTA expression and allow controlled ablation followed by analysis of recovery, [59] although leakiness, recombination efficiency, and transgene mosaicism can limit precision. [60]

ii) Inducible caspase systems: Caspase cascade in apoptosis begins with upstream initiator caspases (e.g., caspase 8) activated by dimerization, which then activates effector caspases (e.g., caspase 3) to execute cellular dismantling. [61-63] In zebrafish, researchers have exploited induction of caspase-8 dimerization to achieve temporal and spatial control of cell-specific ablation in two main ways: 1) activated by the FK1012 chemical inducer of dimerization; [56,64 and, 2) expression of a fusion between caspase and the modified estrogen receptor ligand-binding domain (ERT2), whose activity is induced by binding of tamoxifen. [65-67] Compared to FK1012, tamoxifen pharmacokinetics are better characterized in zebrafish, and a convincing study showing lifelong regeneration of cerebellar Purkinje cells in zebrafish strongly supports the use of the ER-T2/tamoxifen inducible ablation approach for probing cell loss and recovery. [65]

iii) HSV-TK: The herpes simplex virus thymidine kinase (HSV-tk) has been applied in zebrafish for conditional cell ablation. Transgenic expression of this “suicide” gene in a defined cell population converts the antiviral prodrug ganciclovir into toxic nucleotides that are lethal to proliferating cells, but this dependence on cell division has limited the utility of the approach. [68]

iv) Nitroreductase (NTR): Bacterial nitroreductase genes (*nfsB*) are another class of “suicide” genes. These genes encode nitroreductases (NTR), enzymes that convert nitro-containing prodrugs (e.g., nitroimidazoles and nitrofurans) into cytotoxic compounds. As a result, any transgenic zebrafish cell expressing NTR becomes vulnerable to treatment with prodrugs such as metronidazole (MTZ). This approach to cell-specific ablation has become a widely adopted tool in the scientific community. Although first applied as an ablation tool in zebrafish, NTR/MTZ-mediated ablation has

also been adapted for other model organisms such as, NTR/MTZ-mediated ablation has also been adapted for other model organisms; such as: *drosophila*, [69] *nematostella*, [70] *xenopus*, [30,71] medaka, [72] rats [73] and mice. [74] To date in zebrafish, the NTR/MTZ system has been used in a wide variety of different tissue to both study regeneration and model human pathology (**Fig. 1** and **Sup. Table 1**). However, historically, interest in NTR was first driven by its potential as a “suicide gene” for cancer therapy.

Figure 1. Commonly targeted cell types for ablation studies in zebrafish. See S.Table 1 for more complete list. Image of a 5 days post-fertilization (dpf) *Casper* zebrafish larva159 with approximate position of cell type ablated. Name of cell, and transgene provided along with reference. Orange highlights indicate transgenic models used to study human pathologies.

Development of Nitroreductase as a Suicide Gene

Bacterial nitroreductases (NTRs) from *E. coli* (*nfsA* and *nfsB*) were first characterized in the 1970s–80s for their role in reducing nitroaromatics, [75–77] a function later harnessed in the 1990s for Directed Enzyme Prodrug Therapy (DEPT). [78–80] DEPT strategies use viral or antibody-based systems to deliver a ‘suicide gene’ or its enzyme product to tumors (e.g. NTR). [81] Once localized, the enzyme converts an administered prodrug into a cytotoxic agent, selectively killing the cancer cells.

The prodrug CB1954 is harmless to human cells but becomes a powerful, DNA-damaging toxin after activation by NTR. [82,83] Even though the NTR/CB1954 approach has been tested in clinical trials, [84,85] its effectiveness in mitigating cancers is limited due to low NTR activity and slow prodrug metabolism. Despite these therapeutic shortcomings, this work established NTR/CB1954 as a potent conditional cell-killing strategy. However, a key feature of the system, the “bystander effect,” where cytotoxic metabolites diffuse to kill neighboring cells, poses a significant problem for its application in regeneration studies. This effect complicates the interpretation of cell-specific ablation and subsequent regenerative outcomes. To overcome these limitations, researchers turned to alternative prodrugs. Metronidazole (MTZ), a nitroimidazole antibiotic, emerged as a particularly effective option because it is non-toxic to eukaryotic cells until reduced by bacterial NTR. Unlike CB1954, the activated metabolites of MTZ are short-lived and largely confined within the target cell, minimizing bystander effects [86–88]. This property made the NTR/MTZ system especially well-suited for regeneration and developmental studies, where precise, cell-specific ablation is essential.

NTR/MTZ in regenerative studies

The NTR/MTZ system for cell-specific ablation was first described in two 2007 studies. Pisharath et al. placed the *E. coli nfsB* gene (NTR) under the zebrafish insulin promoter to express an NTR–mCherry fusion in pancreatic β cells; treatment with 10 mM MTZ produced complete β -cell loss without affecting neighboring α cells or exocrine tissue. [89] Curado et al. used cell-specific promoters to express CFP–NTR in cardiomyocytes, hepatocytes, and β cells, also showing that MTZ induced targeted cell death with no detectable bystander effects. [90] In each case, tissues regenerated after MTZ withdrawal, establishing the NTR/MTZ system as a versatile, specific, inducible, and reversible tool for regeneration studies, [87,91] with NTR functional whether fused to either the N- or C-terminus of a fluorescent reporter.

Once targeted ablation is established, the next step is to identify the cellular source of regeneration. This is best accomplished by combining ablation with lineage tracing. For example, following the ablation of β -cells or hepatocytes, [89,90] researchers used this approach to trace the origins of new regenerated cells. In both the pancreas and liver, Notch-responsive ductal epithelial cells were identified as facultative progenitors responsible for repopulating the tissue. [92–95] Pancreatic centroacinar cells (CACs) and liver biliary epithelial cells (BECs/cholangiocytes) were shown to delaminate from ducts, dedifferentiate into precursors, proliferate, and then redifferentiate to replenish lost β cells or hepatocytes as needed. Combining single-cell RNA sequencing (scRNA-seq) of pancreatic ducts and hepatic ducts after β -cell/hepatocyte ablation has been used to map molecular mechanisms and identify intermediate progenitor states as new β -cells/hepatocytes are

formed. [96,97] This integrated paradigm of targeted ablation, lineage tracing, and scRNA-seq systematically maps the mechanisms of regeneration, providing a crucial roadmap for developing future regenerative therapies.

NTR/MTZ – Dependent Ablation in Modeling Human Disease

The NTR/MTZ ablation system also lends itself well to modeling human diseases that are characterized by the specific and progressive loss of distinct cell populations. The core strengths of this chemogenetic approach include cell-type specificity, and temporal control which allows it to be a toolkit for recapitulating pathological events *in vivo*. This allows for the real-time dissection of disease initiation, progression, and complex cellular responses to injury. A diverse array of human pathologies has been modeled using NTR/MTZ in zebrafish, including chronic hyperglycemia (a symptom of diabetes), [98] acute liver damage, [92] and cardiac injury. [90,99,100] Here, we analyze selected models in greater detail, focusing on kidney disease, retinal degeneration, demyelinating disorders, and neurodegeneration. (Fig. 1 – orange boxes).

Kidney glomerular disease

Glomerular diseases stem from a common problem: progressive podocyte loss or dysfunction. These cells are crucial for maintaining the kidney's filtration barrier and their damage leads to proteinuria, the leakage of abnormal amounts of protein into the urine. [101] To model this pathology, transgenic zebrafish (e.g., *nphs2:NTR-GFP*) were used, where the *nphs2* promoter drives NTR expression to allow precise, inducible podocyte ablation. [102,103] Administering MTZ triggers rapid podocyte apoptosis, resulting in classic features of human glomerular injury causing disrupted filtration barrier with proteinuria and edema. By mirroring these essential aspects, this model provides a direct and relevant system for studying the progression of human podocytopathies. Furthermore, the zebrafish pronephros enables live imaging of podocyte injury and subsequent regeneration. [102] After MTZ withdrawal, podocyte repopulation occurs through residual cells and local progenitors. [103] This makes the model an ideal platform for investigating the cellular and molecular basis of podocyte repair, with direct relevance for discovering therapeutic pathways in human kidney disease.

Retinal degeneration

Given the high conservation of eye structure between zebrafish and humans, the NTR/MTZ system provides an excellent platform for modeling inherited retinal degenerations such as retinitis pigmentosa and cone dystrophies, conditions in which progressive photoreceptor loss leads to vision decline. [104] By driving NTR expression under photoreceptor-specific promoters, distinct photoreceptor subtypes can be selectively ablated. For example, expression under the rhodopsin (*rho*) promoter enables targeted elimination of rod photoreceptors, providing a robust zebrafish model of retinitis pigmentosa. [12] Similarly, the use of cone opsin promoters such as *opn1sw1* permits ablation of defined cone populations to study cone dystrophies. [105] This targeted ablation triggers apoptotic photoreceptor loss while sparing neighboring retinal cells. [106] A major advantage of the zebrafish system is its capacity for spontaneous retinal regeneration, driven by the dedifferentiation and proliferation of Müller glia that give rise to new photoreceptors. [107,108] The NTR/MTZ paradigm allows for precise initiation and synchronization of this regenerative process, enabling real-time dissection of the cellular and molecular programs underlying photoreceptor replacement and the contributions of innate immune signaling to retinal repair. [109]

Demyelinating disorders

Conventional autoimmune models of Multiple Sclerosis (MS), including Experimental Autoimmune Encephalomyelitis (EAE), often display substantial variability in the timing and severity of disease onset, alongside a highly complex and multifactorial immunopathology. [110] This inherent heterogeneity makes it difficult to disentangle the individual cellular and molecular events that specifically contribute to successful remyelination, thereby limiting the ability to clearly define the mechanisms required for effective tissue repair. To overcome these hurdles, NTR-MTZ based models have been utilized to ablate oligodendrocytes and their progenitors. Tg(*mbp:gal4-vp16*);

Tg(*UAS-E1B:NTR-mCherry*) fish express NTR specifically in mature oligodendrocytes that myelinate CNS axons. Exposure to MTZ in these fish caused rapid and synchronized demyelination within 48 hours, characterized by the retraction of myelin sheaths and oligodendrocyte cell death. [111] Furthermore, subsequent regeneration resulted in myelin sheaths that restored normal length and thickness correlated to axon caliber. [112] This mechanistic parallel is highly relevant, as the failure to restore proper myelin architecture is a central hallmark of progressive disability in human demyelinating diseases. [110]

Dopaminergic neurodegeneration

Traditional genetic models of Parkinson's Disease (PD) often exhibit weak or late-onset phenotypes, while neurotoxin-based models pose significant safety risks to researchers, limiting their scalability for high-content screening. To overcome these hurdles, Kim et al utilized a chemogenetic model in zebrafish to ablate dopaminergic (DA) neurons. [113] NTR expression was driven from the *tyrosine hydroxylase* (*th*) promoter (the *th1* gene encodes an enzyme required for dopamine synthesis). *Th:NTR* fish express NTR1.0 in the DA neurons of the ventral forebrain, the zebrafish homolog of the mammalian substantia nigra. Exposure to MTZ in *th:NTR* fish caused substantial mitochondrial damage in DA neurons, characterized by mtDNA damage, dysfunction, diminished motility, and altered morphology, which ultimately resulted in DA neuron death. [113] This mechanistic parallel is highly relevant, as mitochondrial dysfunction is a central hallmark of human PD pathology. This finding elevates the model from a simple cell-killing assay to one of high pathological relevance, suggesting that the cellular stress induced by the NTR/MTZ system in this context faithfully recapitulates a fundamental aspect of the human disease. Consequently, the system provides a robust and scalable platform uniquely suited for the identification of therapeutic agents using small-molecule screening.

NTR/MTZ-Based Screening

The NTR/MTZ ablation system provides a reproducible and scalable platform for functional screening in zebrafish, combining cell-type specificity, quantitative imaging, and compatibility with both chemical and genetic perturbations (**Fig. 2**). It enables systematic identification of compounds and genes that regulate injury response, cell survival, and regeneration across neural, hepatic, endocrine, and cardiac tissues.

Figure 2. NTR/MTZ-based screening platforms in zebrafish. Overview of the integrated chemogenetic screening workflow using nitroreductase (NTR)-mediated ablation. (A) Experimental design showing transgenic zebrafish expressing NTR in target tissues, baseline imaging, and subsequent metronidazole (MTZ) treatment to induce cell-type-specific ablation. The use of parallel transgenic controls and multiwell plate layout enables quantitative assessment of tissue loss and recovery. (B) High-content chemical screening pipeline integrating automated imaging, hit identification, and pathway-level analysis using standardized statistical metrics. (C) Genetic screening framework coupling sgRNA-based mutagenesis with imaging-based phenotype scoring to uncover modifiers of cell loss or regeneration. (D) Behavioral assays to quantify functional recovery or pharmacological response.

Small-Molecule Screening

The NTR/MTZ ablation system has been adapted for high-content chemical screening in zebrafish, allowing quantitative evaluation of compound effects on cell death, protection, and regeneration across multiple tissues. In the context of retinal degeneration, [114] demonstrated the high-throughput capabilities of the system by screening 2,934 compounds using the *Tg(rho:YFP-NTR)* model of Retinitis Pigmentosa. By driving NTR specifically in rod photoreceptors, the Mumm lab induced targeted cell death and screened for small molecules that could preserve YFP-positive cells despite MTZ exposure. This large-scale effort identified 11 validated neuroprotectants, distinct from simple antioxidants, that were subsequently proven to show conserved efficacy in mouse retinal explant assays. [114] This cross-species validation confirms that the zebrafish NTR system effectively filters for compounds with relevant translational potential for human blindness.

Kim et al. (2022) utilized the *Tg(th:NTR)* model to perform a 1,403-compound screen for Parkinson's disease. By integrating automated imaging with rigorous statistical metrics including the Brain Health Score (BHS) and the Strictly Standardized Mean Difference (SSMD), the researchers identified 57 compounds that preserved dopaminergic neurons (**Figs. 2A-B**). Importantly, the study advanced beyond simple measurements of cell survival and provided mechanistic validation that these compounds protected neurons by restoring mitochondrial function, which is a central hallmark of PD pathology. The predictive validity of these hits was further confirmed through cross-assay validation in a separate Gaucher disease behavior model, demonstrating the system's capacity to identify robust therapeutics for complex neurodegenerative conditions (**Fig. 2D**). [113]

Another promising application of the NTR system lies in the identification of therapeutic agents that actively promote tissue regeneration. Lee et al. (2025) leveraged an optimized QF-based binary expression system (*mbpa:qf2;quas:epNTR-P2A-mCherry*) to perform a remyelination phenotypic screen for regenerative compounds. This transgenic line achieved greater than 85% oligodendrocyte loss following treatment with 2 mM MTZ for 18 hours, creating a highly reproducible regenerative baseline. Using this platform to screen a kinase-inhibitor library, the authors identified the TGF- β receptor I inhibitor AZ-12601011 as a potent driver of remyelination. [115] Mechanistic validation revealed that this compound promotes repair by modulating microglial and progenitor activation, thereby confirming the system's predictive validity for discovering clinically relevant restorative therapeutics that actively drive the reconstruction of functional tissue.

Similar regenerative screens have been successfully implemented in other tissues, such as the pancreas. Andersson et al. (2012) utilized the *Tg(ins:CFP-NTR)* line, crossed with a *Tg(ins:Kaede)* reporter to induce complete β -cell ablation and then monitor the formation of new β cells. This model was used in a high-content screen of approximately 7,000 small molecules to find compounds that would enhance regeneration of the insulin producing β cells. [116] This screen identified adenosine receptor agonists, specifically NECA, as potent stimulators of endocrine regeneration. Detailed mechanistic characterization revealed that NECA signals via the A2aa receptor to specifically enhance the proliferation of regenerating β -cells rather than neogenesis, a therapeutic pathway that was subsequently validated to restore normoglycemia in a streptozotocin-induced diabetic mouse model. [116]

Genetic and CRISPR-Based Screening

Chemical screens can identify potential therapeutic reagents, though their molecular targets often remain unknown. A complementary approach is to perform reverse-genetic screens that integrate NTR-mediated ablation with CRISPR mutagenesis to identify genes affecting regeneration. [117] This mutagenesis is achieved by injecting Cas9 ribonucleoprotein (RNP) complexes multiplexed with several guide RNAs per target gene directly into NTR-transgenic embryos (**Fig. 2C**). This F0 'crispan' strategy generates high-efficiency somatic mutations in the first generation, [118] allowing researchers to induce cell-specific ablation with MTZ and immediately quantify the effect of gene disruption on regeneration without the delay of establishing stable mutant lines.

To identify regulators of Retinal Pigment Epithelium (RPE) repair, Lu et al. (2023) conducted a focused F0 CRISPR screen targeting 27 candidate genes in *rpe65a:nfsB-eGFP* larvae. [119] By injecting ribonucleoprotein (RNP) complexes containing three highly mutagenic guide RNAs per gene, they achieved high-efficiency somatic mutagenesis in F0 injected fish. The NTR/MTZ system induced the synchronized, widespread degeneration of the RPE, which subsequently triggered the secondary loss of photoreceptors. This screen identified numerous regulators of regeneration and revealed a novel mechanism that regulates the infiltration of phagocytic cells required for clearance of debris and complete regeneration. [119]

To find regulators of retinal ganglion cell (RGC) regeneration, Emmerich et al. (2024) performed a large-scale CRISPR screen on 100 genes. Using the *isl2b:Gal4; UAS:YFP-NTR2.0* line for RGC ablation, they identified 18 effector genes comprising key transcription factors and signaling pathway

components. [120] The screen revealed that inhibition of *Ascl1a* accelerated the regeneration of new RGC neurons.

Finally, the integration of F0 mutagenesis with automated imaging establishes a scalable framework for future genetic screens. The 'ZebraReg' platform utilizes a dual-transgenic line (*tbx5a:CreERT2*; *myh7l:loxP-tagBFP-STOP-loxP-mCherry-NTR*) that restricts NTR expression specifically to the heart ventricle. [100] Treatment with MTZ ablated approximately 97% of cardiomyocytes, triggering a robust regenerative response that typically restores the tissue within three days. By combining this precise injury model with F0 CRISPR mutagenesis followed by immediate phenotyping, the study demonstrates a proof-of-concept workflow to understand the genetic mechanisms of cardiac repair.

Caveats and improvements to the NTR/MTZ system

The NTR/MTZ system is widely used for diverse applications, but its performance has varied between labs. Key issues include batch-to-batch and preparation variability of MTZ, the need for high MTZ doses (≈ 10 mM) that can cause off-target toxicity (e.g., developing brain [121], larval/adult intestine [98], and differential susceptibility of some cell types to ablation [91]. Because ablation rate depends on both NTR activity and MTZ dose, researchers have pursued three complementary strategies to improve reproducibility and experimental interpretation: 1) increase NTR expression, 2) engineer higher-activity NTR mutants, and 3) identify more efficacious prodrugs that achieve effective killing at lower, less toxic concentrations. These iterative improvements are aimed at mitigating previous limitations and expanding the range of feasible experimental paradigms and are discussed next:

1) Increase NTR expression: Strong, well-characterized promoters/enhancers (e.g., the zebrafish insulin promoter) [89] can drive high NTR expression, especially when present in multiple copies via Tol2-mediated transgenesis [122]. However, many cell-specific regulatory elements are weak, and maintaining multiple insertions is challenging and prone to genetic drift and intergenerational variability. An alternative is to use a bipartite system such as Gal4/UAS, [123,124] which can produce robust, amplified NTR expression even from single genomic insertion. With this approach, a cell-specific promoter drives a Gal4 transactivator that binds UAS sites to strongly activates *NTR* transcription (**Figure 3**). For example, elements from the 14xUAS constructs of Köster and Fraser [124] were used to generate the transgenic line Tg(UAS-E1B:NTR-mCherry)c264, [91,125]. These fish were distributed by the Zebrafish International Resource Center (ZIRC) and have been widely used by the zebrafish community: 10 of the 32 most-cited papers on zebrafish nitroreductase ablation use this line (**S Table 1**). A caveat is that Gal4/UAS DNA elements can be prone to epigenetic silencing, producing mosaic expression; the repetitive UAS contains multiple CpG sites susceptible to DNA methylation. [126,127] Silencing can be mitigated by using a less repetitive UAS (e.g., 4x) [128] or by using the QF/QUAS bipartite system (derived from *Neurospora*) [129], which has been reported to show reduced silencing [130]. and has recently been adapted for NTR-based ablation (**Figure 3**). [115,131].

Figure 3. Schematic of bipartite systems to drive robust levels of NTR. On left, driver lines lead to expression (dashed arrows) of transactivators (Gal4 or QF, blue, purple spheres) under the control of a regulatory element of interest (REI). These transactivators bind their upstream activating sequences (either UAS or QUAS, green boxes) to achieve controlled and amplified NTR expression (tan spheres) in target cells. NTR expression can be monitored by co-production of mCherry (red spheres) either as a fusion protein with NTR or as separate proteins due to P2A dependent ribosome 'skipping'. [139].

2) Higher-activity NTR mutants: Substantial effort has gone into engineering more active NTRs; first driven by their promise as cancer 'suicide-gene' therapies [132] and later to improve NTR-based ablation in basic research.[133] Two research groups independently engineered the same three substitutions into the wild-type *E. coli* enzyme (now termed NTR1.0), creating more efficient versions they named epNTR and NTR1.1.[132-134]. Cross-species screening identified a highly active nitroreductase (NTR) in *Vibrio vulnificus*. Using this enzyme as a scaffold, rational engineering

yielded the second-generation variant NTR2.0, which exhibits a greater than 100-fold enhancement in activity over the original NTR1.0.[135]

The use of first-generation nitroreductase (NTR1) for chronic cell ablation was problematic, as the required 10 mM metronidazole (MTZ) dose induces intestinal pathology and approaches the LD50 in zebrafish.[98] However, the more active NTR2.0 variant enables effective ablation with far lower, better-tolerated MTZ concentrations. To demonstrate this, Tucker et al. developed a zebrafish model expressing NTR2.0 specifically in pancreatic β cells.[128] They found that efficient larval β cell ablation required only 100 μ M MTZ, a regimen that could be maintained for 10 days without ill effects. In stark contrast, the NTR1 system required a toxic 10 mM MTZ dose, which is lethal to larvae (independent of NTR) within three days. In adult fish, a regimen of 5 mM MTZ for two days followed by two weeks at 1 mM was completely tolerated by wild-type fish with no ill effects but induced sustained hyperglycemia and weight loss in NTR2.0-expressing fish. This established a powerful model for studying chronic diabetic consequences, such as retinopathy, nephropathy, and impaired wound healing.

This well-tolerated ablation paradigm now makes it possible to model a range of other chronic conditions, including neurodegenerative, renal, and muscular disorders. This capability, in turn, facilitates the study of long-term disease progression and the evaluation of new therapeutic interventions.

3) More efficacious prodrugs: Metronidazole (MTZ) efficacy can vary across suppliers and batches. To ensure consistency, it is recommended to prepare fresh MTZ solutions for experiments.[91,98] To overcome MTZ's limitations, alternative prodrugs like nifurpirinol (NFP) have been tested. NFP is a more potent nitrofuranyl-based prodrug.[91,98] However, its structural class is distinct from the nitroimidazole-based prodrugs for which NTR2.0 was specifically engineered.[135] As a nitroimidazole prodrug, Ronidazole (RNZ) likely retains compatibility with newer NTR systems while offering significant practical advantages over MTZ, primarily its better potency.[69,121,136] For instance, in Tg(fabp10:mCherry-NTR) fish, 2 mM RNZ achieved hepatocyte ablation comparable to 10 mM MTZ, a five-fold increase in potency.[136] This pattern of higher efficacy was replicated in a macrophage model, where a five-fold lower RNZ dose was as effective as MTZ.[121] Lai et al. also reported no bystander effects and demonstrated RNZ efficacy with the NTR1.1 variant. It has also been shown that RNZ functions with NTR2.0 to cause cell-specific ablation,[137] although a direct comparison of RNZ versus MTZ with NTR2.0 has not been reported. However, it is anticipated that the NTR2.0/RNZ combination will further lower the required prodrug concentrations and minimize off-target activity.

Given this evolving landscape of prodrugs and enzymes, what are the critical factors a researcher must weigh when designing an NTR ablation experiment?

Experimental Design: Practical and Technical Considerations

A successful ablation experiment using the NTR/MTZ (or RNZ) system will require careful consideration of three key components: 1) transgenic strategy, 2) optimal NTR activity, and 3) appropriate controls. The specific biological question determines the optimal design. For regeneration studies, aim for complete ablation to easily assess for occurrence of neogenesis from progenitor cells. For functional studies, partial loss may be sufficient to observe a phenotype

1) Transgenic strategy: Select regulatory elements carefully for tissue specificity; where a single promoter is insufficient, employ intersectional approaches (e.g., Cre/lox) to restrict NTR expression to the desired cell population.[138] Incorporate an independent fluorescent marker (fusion or 2A reporter[139]) to identify transgenic animals and to confirm cell-type specific expression.

Like any transgene, NTR transgenics may show positional effects such as leakiness and mosaicism, particularly with multicopy insertions. To ensure reliable lines:

- Screen multiple founders. Identify at least five independent F0 founders and ideally establish 5 F1 lines.

- Compare stable F1 lines to confirm that expression of fluorescent marker matches the published characterization of the transgene's regulatory elements.

- Prioritize a subset of F1 lines for further use based on the following criteria:

a) Mendelian transmission consistent with a single-site insertion, which simplifies downstream ablation experiments by avoiding variability from differing copy number.

b) Consistent expression: The linked fluorescent marker should show non-mosaic expression, confirming that NTR is expressed in all intended target cells. Validate by correlating the marker's fluorescence with independent methods like other reporter lines or antibody staining.

c) Robust, reproducible expression that is consistent regardless of whether the transgene is inherited from the male or the female.

2) NTR activity: Often strong NTR expression produces faster, more complete ablation.[98,135] At the moment, NTR2.0 is the most active NTR used in zebrafish and there seems no reason not to use this particular enzyme in future ablation studies. If the promoter to be used to drive NTR2.0 is weak, consider amplifying expression via binary systems (Gal4/UAS or QF/QUAS).[115,125,131]

3) Controls: To allow interpretation of ablation experiments, it is essential to validate that cell death was induced. Standard readouts include apoptosis assays, such as TUNEL and immunostaining for cleaved (active) caspase-3;[87,91] and monitoring of loss of fluorescent reporters.[89-91] If signal perdurance may confound interpretation, consider using destabilized reporters to minimize reporter longevity.[140,141]

A concern with NTR/MTZ ablation is that stressed target cells may downregulate both the NTR enzyme and its fluorescent reporter, allowing these cells to evade prodrug-induced death. While robust NTR expression and optimized dosing mitigate this risk, confirming the kinetics of cell death is often desirable. This can be achieved through endpoint analysis, *i.e.* fixing samples at serial time points and staining for apoptotic markers or by visualizing cell-death biosensors. HMGB1 is a nuclear chromatin-binding protein whose trafficking is death-mode specific: it is passively released during necrosis but exhibits strong nuclear retention during apoptosis.[142,143] By fusing HMGB1 to GFP, it is possible to create a transgenic biosensor that reports on cell death dynamics.[144] In zebrafish larvae co-expressing *ins:mCherry-2a-NTR2.0* and *ins:hmg1-eGFP* in β -cells, [98,145] MTZ treatment triggered apoptosis marked by the loss of cytoplasmic mCherry signal and the concurrent unmasking of nuclei positive for HMGB1-eGFP (**Fig. 4**). A high dose of MTZ (1 mM) induced apoptosis within 4 hours and complete loss of β -cell material by 24 hours, whereas a lower dose (10 μ M) resulted in slower dynamics, with cellular debris still detectable at 24 hours. This approach demonstrates how cell-death reporters can provide feedback on timing and efficiency of cell death, information that is critical for optimizing prodrug regimens and interpreting ablation outcomes.

Figure 4. Live imaging of cell-death kinetics (A) Schematic of 6 dpf larvae showing region of fish imaged in C-N where the pancreatic islet is located (red/yellow). (B) Diagram of the two transgenes (*ins:Hmg1-GFP*, *ins:mCherry-2a-NTR2.0*) in the fish in C-N. The insulin promoter (grey box) drives expression of the following: (B-above) an Hmg1-GFP fusion protein and (B-below) NTR2.0 and mCherry [presence of the P2A (blue box) induces ribosomal skipping producing separate proteins]. C-N Confocal images of the islet of in three larval fish over a time course from 6 dpf to 7 dpf (times along the X axis). C-F negative control – no MTZ (0). G-J fish treated with high MTZ dose (1mM). K-N fish treated with a low dose MTZ (10 μ M). G-N Dying β cells first lose red fluorescence, revealing green nuclei (arrow heads). A higher dose shows appearance of green nuclei (H) earlier than the lower dose (N). J 24 hrs in 1mM and no debris remains.

Finally, in any NTR-based ablation experiment there should be two negative controls:

- NTR transgene, no prodrug - controls for effects of exogenous NTR expression and provides base line response.
- No NTR transgene, prodrug - controls for off-target prodrug effects, including antimicrobial activity (nitroaromatic prodrugs such as MTZ/RNZ will affect the microbiome). For host-microbiome studies, consider alternative ablation methods.

These guidelines are intended to help researchers design robust, interpretable NTR-based ablation experiments, facilitating investigations into important biological questions.

References

- 1 Poss, K. D. Advances in understanding tissue regenerative capacity and mechanisms in animals. *Nat Rev Genet* **11**, 710-722, doi:10.1038/nrg2879 (2010).
- 2 Tanaka, E. M. & Reddien, P. W. The cellular basis for animal regeneration. *Dev Cell* **21**, 172-185, doi:10.1016/j.devcel.2011.06.016 (2011).
- 3 Pellettieri, J. Regenerative tissue remodeling in planarians - The mysteries of morphallaxis. *Semin Cell Dev Biol* **87**, 13-21, doi:10.1016/j.semcdb.2018.04.004 (2019).
- 4 Gurtner, G. C., Werner, S., Barrandon, Y. & Longaker, M. T. Wound repair and regeneration. *Nature* **453**, 314-321, doi:10.1038/nature07039 (2008).
- 5 Jopling, C., Boue, S. & Izpisua Belmonte, J. C. Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. *Nat Rev Mol Cell Biol* **12**, 79-89, doi:10.1038/nrm3043 (2011).
- 6 Tan, F. H. & Bronner, M. E. Regenerative loss in the animal kingdom as viewed from the mouse digit tip and heart. *Dev Biol* **507**, 44-63, doi:10.1016/j.ydbio.2023.12.008 (2024).
- 7 Seifert, A. W. *et al.* Skin shedding and tissue regeneration in African spiny mice (*Acomys*). *Nature* **489**, 561-565, doi:10.1038/nature11499 (2012).
- 8 Patton, E. E., Zon, L. I. & Langenau, D. M. Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. *Nat Rev Drug Discov* **20**, 611-628, doi:10.1038/s41573-021-00210-8 (2021).
- 9 Poss, K. D., Wilson, L. G. & Keating, M. T. Heart regeneration in zebrafish. *Science* **298**, 2188-2190, doi:10.1126/science.1077857 (2002).
- 10 Ross Stewart, K. M., Walker, S. L., Baker, A. H., Riley, P. R. & Brittan, M. Hooked on heart regeneration: the zebrafish guide to recovery. *Cardiovasc Res* **118**, 1667-1679, doi:10.1093/cvr/cvab214 (2022).
- 11 Hammer, J. *et al.* Visual Function is Gradually Restored During Retina Regeneration in Adult Zebrafish. *Front Cell Dev Biol* **9**, 831322, doi:10.3389/fcell.2021.831322 (2021).
- 12 Montgomery, J. E., Parsons, M. J. & Hyde, D. R. A novel model of retinal ablation demonstrates that the extent of rod cell death regulates the origin of the regenerated zebrafish rod photoreceptors. *J Comp Neurol* **518**, 800-814, doi:10.1002/cne.22243 (2010).
- 13 Becker, T., Wullmann, M. F., Becker, C. G., Bernhardt, R. R. & Schachner, M. Axonal regrowth after spinal cord transection in adult zebrafish. *J Comp Neurol* **377**, 577-595, doi:10.1002/(sici)1096-9861(19970127)377:4<577::aid-cne8>3.0.co;2-# (1997).
- 14 Zhou, L. *et al.* Progenitor-derived glia are required for spinal cord regeneration in zebrafish. *Development* **150**, doi:10.1242/dev.201162 (2023).
- 15 Azevedo, A. S., Grotek, B., Jacinto, A., Weidinger, G. & Saude, L. The regenerative capacity of the zebrafish caudal fin is not affected by repeated amputations. *PLoS One* **6**, e22820, doi:10.1371/journal.pone.0022820 (2011).
- 16 Sehring, I. *et al.* Zebrafish fin regeneration involves generic and regeneration-specific osteoblast injury responses. *Elife* **11**, doi:10.7554/eLife.77614 (2022).
- 17 Marques, I. J., Lupi, E. & Mercader, N. Model systems for regeneration: zebrafish. *Development* **146**, doi:10.1242/dev.167692 (2019).
- 18 Pfefferli, C. & Jazwinska, A. The art of fin regeneration in zebrafish. *Regeneration (Oxf)* **2**, 72-83, doi:10.1002/reg.2.33 (2015).
- 19 Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J. & Brand, M. Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. *Development* **138**, 4831-4841, doi:10.1242/dev.072587 (2011).
- 20 Cox, B. D. *et al.* In Toto Imaging of Dynamic Osteoblast Behaviors in Regenerating Skeletal Bone. *Curr Biol* **28**, 3937-3947 e3934, doi:10.1016/j.cub.2018.10.052 (2018).
- 21 Bergen, D. J. M. *et al.* Regenerating zebrafish scales express a subset of evolutionary conserved genes involved in human skeletal disease. *BMC Biol* **20**, 21, doi:10.1186/s12915-021-01209-8 (2022).

- 22 Tsai, S. L., Villaseñor, S., Shah, R. R. & Galloway, J. L. Endogenous tenocyte activation underlies the regenerative capacity of the adult zebrafish tendon. *NPJ Regen Med* **8**, 52, doi:10.1038/s41536-023-00328-w (2023).
- 23 Anderson, T. *et al.* Ligament injury in adult zebrafish triggers ECM remodeling and cell dedifferentiation for scar-free regeneration. *bioRxiv*, doi:10.1101/2023.02.03.527039 (2023).
- 24 Reimer, M. M. *et al.* Motor neuron regeneration in adult zebrafish. *J Neurosci* **28**, 8510-8516, doi:10.1523/JNEUROSCI.1189-08.2008 (2008).
- 25 Goldshmit, Y. *et al.* Fgf-dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. *J Neurosci* **32**, 7477-7492, doi:10.1523/JNEUROSCI.0758-12.2012 (2012).
- 26 Gonzalez-Rosa, J. M. & Mercader, N. Cryoinjury as a myocardial infarction model for the study of cardiac regeneration in the zebrafish. *Nat Protoc* **7**, 782-788, doi:10.1038/nprot.2012.025 (2012).
- 27 Bise, T., Sallin, P., Pfefferli, C. & Jazwinska, A. Multiple cryoinjuries modulate the efficiency of zebrafish heart regeneration. *Sci Rep* **10**, 11551, doi:10.1038/s41598-020-68200-1 (2020).
- 28 Vihtelic, T. S. & Hyde, D. R. Light-induced rod and cone cell death and regeneration in the adult albino zebrafish (*Danio rerio*) retina. *J Neurobiol* **44**, 289-307, doi:10.1002/1097-4695(20000905)44:3<289::aid-neu1>3.0.co;2-h (2000).
- 29 Schuck, J. B. & Smith, M. E. Cell proliferation follows acoustically-induced hair cell bundle loss in the zebrafish saccule. *Hear Res* **253**, 67-76, doi:10.1016/j.heares.2009.03.008 (2009).
- 30 Liang, J. *et al.* The stat3/socs3a pathway is a key regulator of hair cell regeneration in zebrafish. [corrected]. *J Neurosci* **32**, 10662-10673, doi:10.1523/JNEUROSCI.5785-10.2012 (2012).
- 31 Uribe, P. M. *et al.* Larval Zebrafish Lateral Line as a Model for Acoustic Trauma. *eNeuro* **5**, doi:10.1523/ENEURO.0206-18.2018 (2018).
- 32 Liu, K. S. & Fetcho, J. R. Laser ablations reveal functional relationships of segmental hindbrain neurons in zebrafish. *Neuron* **23**, 325-335, doi:10.1016/s0896-6273(00)80783-7 (1999).
- 33 Muto, A. & Kawakami, K. Ablation of a Neuronal Population Using a Two-photon Laser and Its Assessment Using Calcium Imaging and Behavioral Recording in Zebrafish Larvae. *J Vis Exp*, doi:10.3791/57485 (2018).
- 34 Roeser, T. & Baier, H. Visuomotor behaviors in larval zebrafish after GFP-guided laser ablation of the optic tectum. *J Neurosci* **23**, 3726-3734, doi:10.1523/JNEUROSCI.23-09-03726.2003 (2003).
- 35 Matrone, G. *et al.* Laser-targeted ablation of the zebrafish embryonic ventricle: a novel model of cardiac injury and repair. *Int J Cardiol* **168**, 3913-3919, doi:10.1016/j.ijcard.2013.06.063 (2013).
- 36 Miskolci, V. *et al.* Distinct inflammatory and wound healing responses to complex caudal fin injuries of larval zebrafish. *Elife* **8**, doi:10.7554/eLife.45976 (2019).
- 37 Fister, A. M., Horn, A., Lasarev, M. R. & Huttenlocher, A. Damage-induced basal epithelial cell migration modulates the spatial organization of redox signaling and sensory neuron regeneration. *Elife* **13**, doi:10.7554/eLife.94995 (2024).
- 38 Coffin, A. B., Rubel, E. W. & Raible, D. W. Bax, Bcl2, and p53 differentially regulate neomycin- and gentamicin-induced hair cell death in the zebrafish lateral line. *J Assoc Res Otolaryngol* **14**, 645-659, doi:10.1007/s10162-013-0404-1 (2013).
- 39 Uribe, P. M. *et al.* Aminoglycoside-induced hair cell death of inner ear organs causes functional deficits in adult zebrafish (*Danio rerio*). *PLoS One* **8**, e58755, doi:10.1371/journal.pone.0058755 (2013).
- 40 Wiedenhoft, H., Hayashi, L. & Coffin, A. B. PI3K and Inhibitor of Apoptosis Proteins Modulate Gentamicin-Induced Hair Cell Death in the Zebrafish Lateral Line. *Front Cell Neurosci* **11**, 326, doi:10.3389/fncel.2017.00326 (2017).
- 41 Moss, J. B. *et al.* Regeneration of the pancreas in adult zebrafish. *Diabetes* **58**, 1844-1851, doi:10.2337/db08-0628 (2009).
- 42 Fimbel, S. M., Montgomery, J. E., Burket, C. T. & Hyde, D. R. Regeneration of inner retinal neurons after intravitreal injection of ouabain in zebrafish. *J Neurosci* **27**, 1712-1724, doi:10.1523/JNEUROSCI.5317-06.2007 (2007).
- 43 Sherpa, T. *et al.* Ganglion cell regeneration following whole-retina destruction in zebrafish. *Dev Neurobiol* **68**, 166-181, doi:10.1002/dneu.20568 (2008).

- 44 Dovonou, A. *et al.* Animal models of Parkinson's disease: bridging the gap between disease hallmarks and research questions. *Transl Neurodegener* **12**, 36, doi:10.1186/s40035-023-00368-8 (2023).
- 45 Varady, A. & Distel, M. Non-neuromodulatory Optogenetic Tools in Zebrafish. *Front Cell Dev Biol* **8**, 418, doi:10.3389/fcell.2020.00418 (2020).
- 46 Baillie, J. S., Stoyek, M. R. & Quinn, T. A. Seeing the Light: The Use of Zebrafish for Optogenetic Studies of the Heart. *Front Physiol* **12**, 748570, doi:10.3389/fphys.2021.748570 (2021).
- 47 Buckley, C. *et al.* Precise spatio-temporal control of rapid optogenetic cell ablation with mem-KillerRed in Zebrafish. *Sci Rep* **7**, 5096, doi:10.1038/s41598-017-05028-2 (2017).
- 48 Bulina, M. E. *et al.* A genetically encoded photosensitizer. *Nat Biotechnol* **24**, 95-99, doi:10.1038/nbt1175 (2006).
- 49 Teh, C. *et al.* Optogenetic in vivo cell manipulation in KillerRed-expressing zebrafish transgenics. *BMC Dev Biol* **10**, 110, doi:10.1186/1471-213X-10-110 (2010).
- 50 Formella, I. *et al.* Real-time visualization of oxidative stress-mediated neurodegeneration of individual spinal motor neurons in vivo. *Redox Biol* **19**, 226-234, doi:10.1016/j.redox.2018.08.011 (2018).
- 51 Teh, C. & Korzh, V. In vivo optogenetics for light-induced oxidative stress in transgenic zebrafish expressing the KillerRed photosensitizer protein. *Methods Mol Biol* **1148**, 229-238, doi:10.1007/978-1-4939-0470-9_15 (2014).
- 52 Collier, R. J. Understanding the mode of action of diphtheria toxin: a perspective on progress during the 20th century. *Toxicon* **39**, 1793-1803, doi:10.1016/s0041-0101(01)00165-9 (2001).
- 53 Naglich, J. G., Metherall, J. E., Russell, D. W. & Eidels, L. Expression cloning of a diphtheria toxin receptor: identity with a heparin-binding EGF-like growth factor precursor. *Cell* **69**, 1051-1061, doi:10.1016/0092-8674(92)90623-k (1992).
- 54 Saito, M. *et al.* Diphtheria toxin receptor-mediated conditional and targeted cell ablation in transgenic mice. *Nat Biotechnol* **19**, 746-750, doi:10.1038/90795 (2001).
- 55 Jimenez, E., Slevin, C. C., Colon-Cruz, L. & Burgess, S. M. Vestibular and Auditory Hair Cell Regeneration Following Targeted Ablation of Hair Cells With Diphtheria Toxin in Zebrafish. *Front Cell Neurosci* **15**, 721950, doi:10.3389/fncel.2021.721950 (2021).
- 56 Schmitner, N., Kohno, K. & Meyer, D. ptf1a(+) , ela3l(-) cells are developmentally maintained progenitors for exocrine regeneration following extreme loss of acinar cells in zebrafish larvae. *Dis Model Mech* **10**, 307-321, doi:10.1242/dmm.026633 (2017).
- 57 Kurita, R. *et al.* Suppression of lens growth by alphaA-crystallin promoter-driven expression of diphtheria toxin results in disruption of retinal cell organization in zebrafish. *Dev Biol* **255**, 113-127, doi:10.1016/s0012-1606(02)00079-9 (2003).
- 58 Li, Z., Korzh, V. & Gong, Z. DTA-mediated targeted ablation revealed differential interdependence of endocrine cell lineages in early development of zebrafish pancreas. *Differentiation* **78**, 241-252, doi:10.1016/j.diff.2009.05.009 (2009).
- 59 Sun, F., Shoffner, A. R. & Poss, K. D. A Genetic Cardiomyocyte Ablation Model for the Study of Heart Regeneration in Zebrafish. *Methods Mol Biol* **2158**, 71-80, doi:10.1007/978-1-0716-0668-1_7 (2021).
- 60 Erhardt, V. *et al.* Systematic optimization and prediction of cre recombinase for precise genome editing in mice. *Genome Biol* **26**, 85, doi:10.1186/s13059-025-03560-3 (2025).
- 61 Salvesen, G. S. & Dixit, V. M. Caspases: intracellular signaling by proteolysis. *Cell* **91**, 443-446, doi:10.1016/s0092-8674(00)80430-4 (1997).
- 62 Boatright, K. M. & Salvesen, G. S. Mechanisms of caspase activation. *Curr Opin Cell Biol* **15**, 725-731, doi:10.1016/j.ceb.2003.10.009 (2003).
- 63 Riedl, S. J. & Salvesen, G. S. The apoptosome: signalling platform of cell death. *Nat Rev Mol Cell Biol* **8**, 405-413, doi:10.1038/nrm2153 (2007).
- 64 Banaszynski, L. A., Chen, L. C., Maynard-Smith, L. A., Ooi, A. G. & Wandless, T. J. A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell* **126**, 995-1004, doi:10.1016/j.cell.2006.07.025 (2006).
- 65 Pose-Mendez, S. *et al.* Lifelong regeneration of cerebellar Purkinje cells after induced cell ablation in zebrafish. *Elife* **12**, doi:10.7554/eLife.79672 (2023).

- 66 Weber, T. *et al.* Caspase-mediated apoptosis induction in zebrafish cerebellar Purkinje neurons. *Development* **143**, 4279-4287, doi:10.1242/dev.122721 (2016).
- 67 Chu, Y., Senghaas, N., Koster, R. W., Wurst, W. & Kuhn, R. Novel caspase-suicide proteins for tamoxifen-inducible apoptosis. *Genesis* **46**, 530-536, doi:10.1002/dvg.20426 (2008).
- 68 Moro, E., Gnugge, L., Braghetta, P., Bortolussi, M. & Argenton, F. Analysis of beta cell proliferation dynamics in zebrafish. *Dev Biol* **332**, 299-308, doi:10.1016/j.ydbio.2009.05.576 (2009).
- 69 Teeters, G., Cucolo, C. E., Kasar, S. N., Worley, M. I. & Siegrist, S. E. Spatiotemporal control of cell ablation using Ronidazole with Nitroreductase in *Drosophila*. *Dev Biol* **520**, 31-40, doi:10.1016/j.ydbio.2024.12.017 (2025).
- 70 Mazloumi Gavgani, F. *et al.* Ectopic head regeneration after nervous system ablation in a sea anemone. *Curr Biol* **35**, 5955-5964 e5953, doi:10.1016/j.cub.2025.10.061 (2025).
- 71 Mannioui, A. *et al.* The *Xenopus* tadpole: An in vivo model to screen drugs favoring remyelination. *Mult Scler* **24**, 1421-1432, doi:10.1177/1352458517721355 (2018).
- 72 Willems, B. *et al.* Conditional ablation of osteoblasts in medaka. *Dev Biol* **364**, 128-137, doi:10.1016/j.ydbio.2012.01.023 (2012).
- 73 Kwak, S. P. *et al.* Ablation of central nervous system progenitor cells in transgenic rats using bacterial nitroreductase system. *J Neurosci Res* **85**, 1183-1193, doi:10.1002/jnr.21223 (2007).
- 74 Isles, A. R. *et al.* Conditional ablation of neurones in transgenic mice. *J Neurobiol* **47**, 183-193, doi:10.1002/neu.1026 (2001).
- 75 Bryant, D. W., McCalla, D. R., Leeksa, M. & Laneville, P. Type I nitroreductases of *Escherichia coli*. *Can J Microbiol* **27**, 81-86, doi:10.1139/m81-013 (1981).
- 76 Whiteway, J. *et al.* Oxygen-insensitive nitroreductases: analysis of the roles of *nfsA* and *nfsB* in development of resistance to 5-nitrofur derivatives in *Escherichia coli*. *J Bacteriol* **180**, 5529-5539, doi:10.1128/JB.180.21.5529-5539.1998 (1998).
- 77 McCalla, D. R., Kaiser, C. & Green, M. H. Genetics of nitrofurazone resistance in *Escherichia coli*. *J Bacteriol* **133**, 10-16, doi:10.1128/jb.133.1.10-16.1978 (1978).
- 78 Zawilska, J. B., Wojcieszak, J. & Olejniczak, A. B. Prodrugs: a challenge for the drug development. *Pharmacol Rep* **65**, 1-14, doi:10.1016/s1734-1140(13)70959-9 (2013).
- 79 Karjoo, Z., Chen, X. & Hatefi, A. Progress and problems with the use of suicide genes for targeted cancer therapy. *Adv Drug Deliv Rev* **99**, 113-128, doi:10.1016/j.addr.2015.05.009 (2016).
- 80 Drabek, D., Guy, J., Craig, R. & Grosveld, F. The expression of bacterial nitroreductase in transgenic mice results in specific cell killing by the prodrug CB1954. *Gene Ther* **4**, 93-100, doi:10.1038/sj.gt.3300366 (1997).
- 81 Knox, R. J., Friedlos, F. & Boland, M. P. The bioactivation of CB 1954 and its use as a prodrug in antibody-directed enzyme prodrug therapy (ADEPT). *Cancer Metastasis Rev* **12**, 195-212, doi:10.1007/BF00689810 (1993).
- 82 Knox, R. J. *et al.* The nitroreductase enzyme in Walker cells that activates 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) to 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide is a form of NAD(P)H dehydrogenase (quinone) (EC 1.6.99.2). *Biochem Pharmacol* **37**, 4671-4677, doi:10.1016/0006-2952(88)90336-x (1988).
- 83 Anlezark, G. M. *et al.* Bioactivation of dinitrobenzamide mustards by an *E. coli* B nitroreductase. *Biochem Pharmacol* **50**, 609-618, doi:10.1016/0006-2952(95)00187-5 (1995).
- 84 Palmer, D. H. *et al.* Virus-directed enzyme prodrug therapy: intratumoral administration of a replication-deficient adenovirus encoding nitroreductase to patients with resectable liver cancer. *J Clin Oncol* **22**, 1546-1552, doi:10.1200/JCO.2004.10.005 (2004).
- 85 Patel, P. *et al.* A phase I/II clinical trial in localized prostate cancer of an adenovirus expressing nitroreductase with CB1954 [correction of CB1984]. *Mol Ther* **17**, 1292-1299, doi:10.1038/mt.2009.80 (2009).
- 86 Bridgewater, J. A., Knox, R. J., Pitts, J. D., Collins, M. K. & Springer, C. J. The bystander effect of the nitroreductase/CB1954 enzyme/prodrug system is due to a cell-permeable metabolite. *Hum Gene Ther* **8**, 709-717, doi:10.1089/hum.1997.8.6-709 (1997).

- 87 Curado, S., Stainier, D. Y. & Anderson, R. M. Nitroreductase-mediated cell/tissue ablation in zebrafish: a spatially and temporally controlled ablation method with applications in developmental and regeneration studies. *Nat Protoc* **3**, 948-954, doi:10.1038/nprot.2008.58 (2008).
- 88 Sharrock, A. V. *et al.* Engineering the Escherichia coli Nitroreductase NfsA to Create a Flexible Enzyme-Prodrug Activation System. *Front Pharmacol* **12**, 701456, doi:10.3389/fphar.2021.701456 (2021).
- 89 Pisharath, H., Rhee, J. M., Swanson, M. A., Leach, S. D. & Parsons, M. J. Targeted ablation of beta cells in the embryonic zebrafish pancreas using E. coli nitroreductase. *Mech Dev* **124**, 218-229, doi:10.1016/j.mod.2006.11.005 (2007).
- 90 Curado, S. *et al.* Conditional targeted cell ablation in zebrafish: a new tool for regeneration studies. *Dev Dyn* **236**, 1025-1035, doi:10.1002/dvdy.21100 (2007).
- 91 Pisharath, H. & Parsons, M. J. Nitroreductase-mediated cell ablation in transgenic zebrafish embryos. *Methods Mol Biol* **546**, 133-143, doi:10.1007/978-1-60327-977-2_9 (2009).
- 92 Choi, T. Y., Ninov, N., Stainier, D. Y. & Shin, D. Extensive conversion of hepatic biliary epithelial cells to hepatocytes after near total loss of hepatocytes in zebrafish. *Gastroenterology* **146**, 776-788, doi:10.1053/j.gastro.2013.10.019 (2014).
- 93 He, J., Lu, H., Zou, Q. & Luo, L. Regeneration of liver after extreme hepatocyte loss occurs mainly via biliary transdifferentiation in zebrafish. *Gastroenterology* **146**, 789-800 e788, doi:10.1053/j.gastro.2013.11.045 (2014).
- 94 Delaspre, F. *et al.* Centroacinar Cells Are Progenitors That Contribute to Endocrine Pancreas Regeneration. *Diabetes* **64**, 3499-3509, doi:10.2337/db15-0153 (2015).
- 95 Ghaye, A. P. *et al.* Progenitor potential of nkx6.1-expressing cells throughout zebrafish life and during beta cell regeneration. *BMC Biol* **13**, 70, doi:10.1186/s12915-015-0179-4 (2015).
- 96 Mi, J., Liu, K. C. & Andersson, O. Decoding pancreatic endocrine cell differentiation and beta cell regeneration in zebrafish. *Sci Adv* **9**, eadf5142, doi:10.1126/sciadv.adf5142 (2023).
- 97 Eski, S. E. *et al.* Cholangiocytes contribute to hepatocyte regeneration after partial liver injury during growth spurt in zebrafish. *Nat Commun* **16**, 5260, doi:10.1038/s41467-025-60334-y (2025).
- 98 Tucker, T. R. *et al.* An inducible model of chronic hyperglycemia. *Dis Model Mech* **16**, doi:10.1242/dmm.050215 (2023).
- 99 Palencia-Desai, S. *et al.* Myocardium and BMP signaling are required for endocardial differentiation. *Development* **142**, 2304-2315, doi:10.1242/dev.118687 (2015).
- 100 Apolinova, K. *et al.* ZebraReg-a novel platform for discovering regulators of cardiac regeneration using zebrafish. *Front Cell Dev Biol* **12**, 1384423, doi:10.3389/fcell.2024.1384423 (2024).
- 101 Greka, A. & Mundel, P. Cell biology and pathology of podocytes. *Annu Rev Physiol* **74**, 299-323, doi:10.1146/annurev-physiol-020911-153238 (2012).
- 102 Zhou, W. & Hildebrandt, F. Inducible podocyte injury and proteinuria in transgenic zebrafish. *J Am Soc Nephrol* **23**, 1039-1047, doi:10.1681/ASN.2011080776 (2012).
- 103 Huang, J. *et al.* A zebrafish model of conditional targeted podocyte ablation and regeneration. *Kidney Int* **83**, 1193-1200, doi:10.1038/ki.2013.6 (2013).
- 104 Narayan, D. S., Wood, J. P., Chidlow, G. & Casson, R. J. A review of the mechanisms of cone degeneration in retinitis pigmentosa. *Acta Ophthalmol* **94**, 748-754, doi:10.1111/aos.13141 (2016).
- 105 Fraser, B., DuVal, M. G., Wang, H. & Allison, W. T. Regeneration of cone photoreceptors when cell ablation is primarily restricted to a particular cone subtype. *PLoS One* **8**, e55410, doi:10.1371/journal.pone.0055410 (2013).
- 106 White, D. T. *et al.* Immunomodulation-accelerated neuronal regeneration following selective rod photoreceptor cell ablation in the zebrafish retina. *Proc Natl Acad Sci U S A* **114**, E3719-E3728, doi:10.1073/pnas.1617721114 (2017).
- 107 Bernardos, R. L., Barthel, L. K., Meyers, J. R. & Raymond, P. A. Late-stage neuronal progenitors in the retina are radial Muller glia that function as retinal stem cells. *J Neurosci* **27**, 7028-7040, doi:10.1523/JNEUROSCI.1624-07.2007 (2007).
- 108 Fausett, B. V. & Goldman, D. A role for alpha1 tubulin-expressing Muller glia in regeneration of the injured zebrafish retina. *J Neurosci* **26**, 6303-6313, doi:10.1523/JNEUROSCI.0332-06.2006 (2006).

109 Langhe, R. *et al.* Muller glial cell reactivation in *Xenopus* models of retinal degeneration. *Glia* **65**, 1333-1349, doi:10.1002/glia.23165 (2017).

110 Constantinescu, C. S., Farooqi, N., O'Brien, K. & Gran, B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol* **164**, 1079-1106, doi:10.1111/j.1476-5381.2011.01302.x (2011).

111 Chung, A. Y. *et al.* Generation of demyelination models by targeted ablation of oligodendrocytes in the zebrafish CNS. *Mol Cells* **36**, 82-87, doi:10.1007/s10059-013-0087-9 (2013).

112 Karttunen, M. J., Czopka, T., Goedhart, M., Early, J. J. & Lyons, D. A. Regeneration of myelin sheaths of normal length and thickness in the zebrafish CNS correlates with growth of axons in caliber. *PLoS One* **12**, e0178058, doi:10.1371/journal.pone.0178058 (2017).

113 Kim, G. J. *et al.* A zebrafish screen reveals Renin-angiotensin system inhibitors as neuroprotective via mitochondrial restoration in dopamine neurons. *Elife* **10**, doi:10.7554/eLife.69795 (2021).

114 Zhang, L. *et al.* Large-scale phenotypic drug screen identifies neuroprotectants in zebrafish and mouse models of retinitis pigmentosa. *Elife* **10**, doi:10.7554/eLife.57245 (2021).

115 Lee, Y. *et al.* Transforming growth factor-beta receptor I kinase plays a crucial role in oligodendrocyte regeneration after demyelination. *Biomed Pharmacother* **187**, 118094, doi:10.1016/j.biopha.2025.118094 (2025).

116 Andersson, O. *et al.* Adenosine signaling promotes regeneration of pancreatic beta cells in vivo. *Cell Metab* **15**, 885-894, doi:10.1016/j.cmet.2012.04.018 (2012).

117 Unal Eroglu, A. *et al.* Multiplexed CRISPR/Cas9 Targeting of Genes Implicated in Retinal Regeneration and Degeneration. *Front Cell Dev Biol* **6**, 88, doi:10.3389/fcell.2018.00088 (2018).

118 Wu, R. S. *et al.* A Rapid Method for Directed Gene Knockout for Screening in G0 Zebrafish. *Dev Cell* **46**, 112-125 e114, doi:10.1016/j.devcel.2018.06.003 (2018).

119 Lu, F., Leach, L. L. & Gross, J. M. A CRISPR-Cas9-mediated F0 screen to identify pro-regenerative genes in the zebrafish retinal pigment epithelium. *Sci Rep* **13**, 3142, doi:10.1038/s41598-023-29046-5 (2023).

120 Emmerich, K. *et al.* A large-scale CRISPR screen reveals context-specific genetic regulation of retinal ganglion cell regeneration. *Development* **151**, doi:10.1242/dev.202754 (2024).

121 Lai, S. *et al.* Chemical screening reveals Ronidazole is a superior prodrug to Metronidazole for nitroreductase-induced cell ablation system in zebrafish larvae. *J Genet Genomics* **48**, 1081-1090, doi:10.1016/j.jgg.2021.07.015 (2021).

122 Kalvaityte, M., Gabrilaviciute, S. & Balciunas, D. Rapid generation of single-insertion transgenics by Tol2 transposition in zebrafish. *Dev Dyn* **253**, 1056-1065, doi:10.1002/dvdy.719 (2024).

123 Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415, doi:10.1242/dev.118.2.401 (1993).

124 Koster, R. W. & Fraser, S. E. Tracing transgene expression in living zebrafish embryos. *Dev Biol* **233**, 329-346, doi:10.1006/dbio.2001.0242 (2001).

125 Davison, J. M. *et al.* Transactivation from Gal4-VP16 transgenic insertions for tissue-specific cell labeling and ablation in zebrafish. *Dev Biol* **304**, 811-824, doi:10.1016/j.ydbio.2007.01.033 (2007).

126 Halpern, M. E. *et al.* Gal4/UAS transgenic tools and their application to zebrafish. *Zebrafish* **5**, 97-110, doi:10.1089/zeb.2008.0530 (2008).

127 Goll, M. G., Anderson, R., Stainier, D. Y., Spradling, A. C. & Halpern, M. E. Transcriptional silencing and reactivation in transgenic zebrafish. *Genetics* **182**, 747-755, doi:10.1534/genetics.109.102079 (2009).

128 Akitake, C. M., Macurak, M., Halpern, M. E. & Goll, M. G. Transgenerational analysis of transcriptional silencing in zebrafish. *Dev Biol* **352**, 191-201, doi:10.1016/j.ydbio.2011.01.002 (2011).

129 Potter, C. J., Tasic, B., Russler, E. V., Liang, L. & Luo, L. The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. *Cell* **141**, 536-548, doi:10.1016/j.cell.2010.02.025 (2010).

130 Subedi, A. *et al.* Adoption of the Q transcriptional regulatory system for zebrafish transgenesis. *Methods* **66**, 433-440, doi:10.1016/j.ymeth.2013.06.012 (2014).

131 Lengyel, M. *et al.* The G-protein coupled receptor OXER1 is a tissue redox sensor essential for intestinal epithelial barrier integrity. *bioRxiv*, doi:10.1101/2025.02.05.636712 (2025).

- 132 Guise, C. P., Grove, J. I., Hyde, E. I. & Searle, P. F. Direct positive selection for improved nitroreductase variants using SOS triggering of bacteriophage lambda lytic cycle. *Gene Ther* **14**, 690-698, doi:10.1038/sj.gt.3302919 (2007).
- 133 Mathias, J. R., Zhang, Z., Saxena, M. T. & Mumm, J. S. Enhanced cell-specific ablation in zebrafish using a triple mutant of Escherichia coli nitroreductase. *Zebrafish* **11**, 85-97, doi:10.1089/zeb.2013.0937 (2014).
- 134 Tabor, K. M. *et al.* Direct activation of the Mauthner cell by electric field pulses drives ultrarapid escape responses. *J Neurophysiol* **112**, 834-844, doi:10.1152/jn.00228.2014 (2014).
- 135 Sharrock, A. V. *et al.* NTR 2.0: a rationally engineered prodrug-converting enzyme with substantially enhanced efficacy for targeted cell ablation. *Nat Methods* **19**, 205-215, doi:10.1038/s41592-021-01364-4 (2022).
- 136 Chen, Y. *et al.* Ronidazole Is a Superior Prodrug to Metronidazole for Nitroreductase-Mediated Hepatocytes Ablation in Zebrafish Larvae. *Zebrafish* **20**, 95-102, doi:10.1089/zeb.2022.0066 (2023).
- 137 Duan, Z. *et al.* Chemogenetic ablation and regeneration of arterial valve in zebrafish. *Biochem Biophys Res Commun* **762**, 151786, doi:10.1016/j.bbrc.2025.151786 (2025).
- 138 Zhong, Y. *et al.* Improved Tol2-mediated enhancer trap identifies weakly expressed genes during liver and beta cell development and regeneration in zebrafish. *J Biol Chem* **294**, 932-940, doi:10.1074/jbc.RA118.005568 (2019).
- 139 Provost, E., Rhee, J. & Leach, S. D. Viral 2A peptides allow expression of multiple proteins from a single ORF in transgenic zebrafish embryos. *Genesis* **45**, 625-629, doi:10.1002/dvg.20338 (2007).
- 140 Walker, S. L. *et al.* Automated reporter quantification in vivo: high-throughput screening method for reporter-based assays in zebrafish. *PLoS One* **7**, e29916, doi:10.1371/journal.pone.0029916 (2012).
- 141 Wang, G. *et al.* First quantitative high-throughput screen in zebrafish identifies novel pathways for increasing pancreatic beta-cell mass. *Elife* **4**, doi:10.7554/eLife.08261 (2015).
- 142 Raucchi, A., Palumbo, R. & Bianchi, M. E. HMGB1: a signal of necrosis. *Autoimmunity* **40**, 285-289, doi:10.1080/08916930701356978 (2007).
- 143 Scaffidi, P., Misteli, T. & Bianchi, M. E. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* **418**, 191-195, doi:10.1038/nature00858 (2002).
- 144 Martins, I. *et al.* Fluorescent biosensors for the detection of HMGB1 release. *Methods Mol Biol* **1004**, 43-56, doi:10.1007/978-1-62703-383-1_4 (2013).
- 145 Parsons, M. J. *et al.* Notch-responsive cells initiate the secondary transition in larval zebrafish pancreas. *Mech Dev* **126**, 898-912, doi:10.1016/j.mod.2009.07.002 (2009).
- 146 Ohnmacht, J. *et al.* Spinal motor neurons are regenerated after mechanical lesion and genetic ablation in larval zebrafish. *Development* **143**, 1464-1474, doi:10.1242/dev.129155 (2016).
- 147 Johnson, K. *et al.* Gfap-positive radial glial cells are an essential progenitor population for later-born neurons and glia in the zebrafish spinal cord. *Glia* **64**, 1170-1189, doi:10.1002/glia.22990 (2016).
- 148 Zhao, X. F., Ellingsen, S. & Fjose, A. Labelling and targeted ablation of specific bipolar cell types in the zebrafish retina. *BMC Neurosci* **10**, 107, doi:10.1186/1471-2202-10-107 (2009).
- 149 Pipalia, T. G. *et al.* Cellular dynamics of regeneration reveals role of two distinct Pax7 stem cell populations in larval zebrafish muscle repair. *Dis Model Mech* **9**, 671-684, doi:10.1242/dmm.022251 (2016).
- 150 Godoy, R., Noble, S., Yoon, K., Anisman, H. & Ekker, M. Chemogenetic ablation of dopaminergic neurons leads to transient locomotor impairments in zebrafish larvae. *J Neurochem* **135**, 249-260, doi:10.1111/jnc.13214 (2015).
- 151 Chen, C. F. *et al.* Establishment of a transgenic zebrafish line for superficial skin ablation and functional validation of apoptosis modulators in vivo. *PLoS One* **6**, e20654, doi:10.1371/journal.pone.0020654 (2011).
- 152 Hanovice, N. J. *et al.* Regeneration of the zebrafish retinal pigment epithelium after widespread genetic ablation. *PLoS Genet* **15**, e1007939, doi:10.1371/journal.pgen.1007939 (2019).
- 153 Dai, X., Jin, X., Chen, X., He, J. & Yin, Z. Sufficient numbers of early germ cells are essential for female sex development in zebrafish. *PLoS One* **10**, e0117824, doi:10.1371/journal.pone.0117824 (2015).
- 154 Hsu, C. C., Hou, M. F., Hong, J. R., Wu, J. L. & Her, G. M. Inducible male infertility by targeted cell ablation in zebrafish testis. *Mar Biotechnol (NY)* **12**, 466-478, doi:10.1007/s10126-009-9248-4 (2010).
- 155 Hu, S. Y. *et al.* Nitroreductase-mediated gonadal dysgenesis for infertility control of genetically modified zebrafish. *Mar Biotechnol (NY)* **12**, 569-578, doi:10.1007/s10126-009-9244-8 (2010).

- 156 White, Y. A., Woods, D. C. & Wood, A. W. A transgenic zebrafish model of targeted oocyte ablation and de novo oogenesis. *Dev Dyn* **240**, 1929-1937, doi:10.1002/dvdy.22695 (2011).
- 157 Kulkarni, A. A. *et al.* An In Vivo Zebrafish Model for Interrogating ROS-Mediated Pancreatic beta-Cell Injury, Response, and Prevention. *Oxid Med Cell Longev* **2018**, 1324739, doi:10.1155/2018/1324739 (2018).
- 158 Li, X. *et al.* Pineal photoreceptor cells are required for maintaining the circadian rhythms of behavioral visual sensitivity in zebrafish. *PLoS One* **7**, e40508, doi:10.1371/journal.pone.0040508 (2012).
- 159 White, R. M. *et al.* Transparent adult zebrafish as a tool for in vivo transplantation analysis. *Cell Stem Cell* **2**, 183-189, doi:10.1016/j.stem.2007.11.002 (2008).

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