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REVIEW

Microscopic modulation and analysis of islets of Langerhans in living zebrafish larvae

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Microscopic analysis of molecules and physiology in living cells and systems is a powerful tool in life sciences. While *in vivo* subcellular microscopic analysis of healthy and diseased human organs remains impossible, zebrafish larvae allow studying pathophysiology of many organs using *in vivo* microscopy. Here, we review the potential of the larval zebrafish pancreas in the context of islets of Langerhans and Type 1 diabetes. We highlight the match of zebrafish larvae with the expanding toolbox of fluorescent probes that monitor cell identity, fate and/or physiology in real time. Moreover, fast and efficient modulation and localization of fluorescence at a subcellular level, through fluorescence microscopy, including confocal and light sheet (single plane illumination) microscopes tailored to *in vivo* larval research, is addressed. These developments make the zebrafish larvae an extremely powerful research tool for translational research. We foresee that living larval zebrafish models will replace many cell line-based studies in understanding the contribution of molecules, organelles and cells to organ pathophysiology in whole organisms.

Keywords: ablation; beta cell stress; endocrine cells; exocrine cells; fluorescent reporters; microscopy; optogenetics; single plane illumination; Type 1 diabetes; zebrafish

Introduction

Microscopy stood at the core of Paul Langerhans's thesis (1869) on the anatomy of the pancreas, including the first description of pancreatic cell clusters now known as the islets of Langerhans [1]. Two decades later, the removal of the pancreas in dogs was associated with diabetes mellitus symptoms and more recently also demonstrated in zebrafish [2,3]. Administration of extracts of islets of Langerhans rescued this phenotype in both depancreatized animals as well as in diabetic patients [4,5]. The century-old discovery that connected Type 1 diabetes (T1D) to islets of Langerhans ultimately led to the current life-saving insulin therapy. Moreover, (immuno)histological analysis revealed inflammatory infiltrates in the islets of Langerhans as well as a decrease of the insulin-producing beta cells in T1D patients [6,7]. While much has been learned regarding the genetic predisposition and auto-immune attack in T1D, as well as its secondary complications, the trigger(s) initiating T1D remain unknown. With currently no long-term alternative treatment to insulin therapy, no cure nor prevention available, fundamental events on the *in vivo* dynamics of T1D onset and beta cells need to be unravelled. However, this cannot be done in humans

Abbreviations
CLSM, confocal laser scanning microscopy; DTA, diphtheria toxin A; ER, endoplasmic reticulum; FP, fluorescent protein; GFP, green fluorescent protein; hpf, hours post-fertilization; MPE, multi-photon excitation; MTZ, metronidazole; NTR, nitroreductase; SPIM, single-plane illumination microscopy; STZ, streptozotocin; T1D, Type 1 diabetes.

[8] but various rodent animal models, such as mice and rats [9], have been widely used in T1D research. Yet, visualizing dynamic biological events *in vivo* in both space and time has remained a complex procedure. Indeed, human cultured cell lines or more sophisticated human organoids and stem cell models [10,11] are more suitable to microscopic interventions and may reflect the human cell physiology more closely, but they lack the cellular context of a multicellular organism. Zebrafish (*Danio rerio*) larvae have great potential for studying the basic physiology of beta cell niches *in vivo* and *in situ*, and their vulnerability to modulated stressors, as reviewed below.

Early larval zebrafish as a vertebrate model organism

Zebrafish larvae are used as a vertebrate *in vivo* model to study fundamentals of organ development and malfunction [12]. Humans and zebrafish share a high degree of similarity in both their organ systems and genetic markup, where 70% of the human protein-coding genes have one or more orthologous genes in zebrafish [13]. Externally fertilized offspring are readily available in high amounts and at relatively low cost, thereby allowing high-throughput research. Additionally, zebrafish larvae up to 120 hours post-fertilization (hpf) have another key advantage, that is being excluded from regulations regarding animal experiments in the European Union [14].

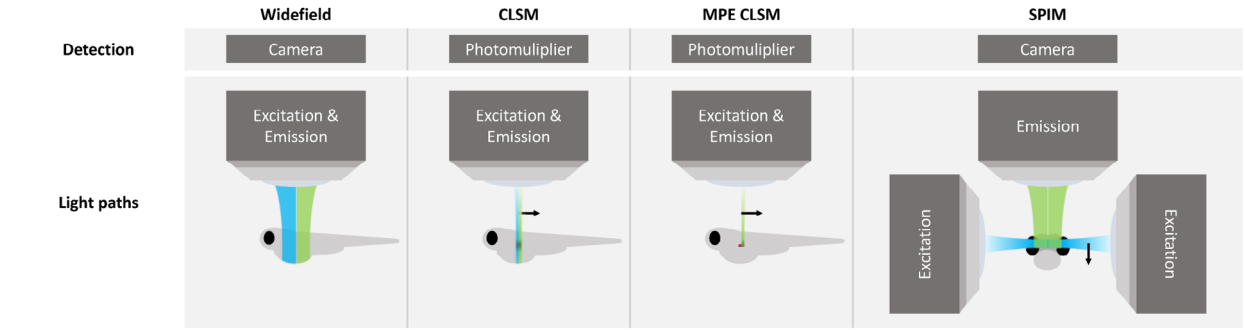
Genetic modifications are easily made in the zebrafish, ranging from transient knock-downs to transgenic models (reviewed in ref. [15]). Random incorporation of exogenous DNA into the zebrafish genome of embryos is efficiently achieved by transposon-based systems, such as *Tol2* [16,17]. The resulting expression of the transgene can be analysed directly in the larvae, or fish can be grown to adulthood to create stable transgenic lines. Tissue-specific promoters are utilized to restrict the expression of proteins to the tissue of interest. When more targeted editing strategies are preferred, a specific nuclease like ZFN (for the zinc finger nuclease), TALEN (for transcription activator-like effector nuclease) or CRISPR/Cas9-based systems are used, as excellently reviewed previously [15,18]. The small size (millimetres) and transparency in the early larval stages, together with the aforementioned benefits, make the zebrafish an excellent model for the visualization of dynamic biological processes at a (sub)cellular level *in vivo* in vertebrates. The ongoing developments in fluorescence microscopy techniques and probes aid to analyse physiology and biodynamics in zebrafish.

Fluorescence microscopy has revolutionized *in vivo* imaging

Fluorescent proteins (FPs) have revolutionized the way researchers view and track dynamic cellular events in living cells and animals. The first reported application of the GFP *in vivo* was by expression of the GFP under the promotor of the *mec-7* gene in *Caenorhabditis elegans* leading to fluorescently labelled touch receptor neurons [19]. Ever since, the toolbox of fluorescent probes [20] has been expanding, not only leading to more photostable probes in diverse colours but also leading to more efficient, less harmful and more imaging-compatible FPs for living systems. Moreover, (optogenetic) modulators and fluorescent reporters of biological activity have been developed and are shared by several labs, for example, via Addgene.org.

Because the early zebrafish are both small and translucent, they are well suited for fluorescent microscopy analysis, which can be performed using different approaches, each with its own benefits and limitations (Fig. 1). Widefield fluorescence microscopy allows fast assessment, but whole specimen illumination negatively impacts resolution and the signal-to-noise ratio due to out-of-focus emission [21]. Resolution is improved in single-photon confocal laser-scanning microscopy (CLSM) by pinholes that block out-of-focus emission light [21]. Multi-photon excitation (MPE) with larger wavelength photons allows deeper imaging with excitation only occurring in the focal point due to the intensity-dependent arrival of multiple photons simultaneously needed to excite the fluorophore (reviewed in [22]). However, acquisition speed in laser scanning microscopy is slow due to the analysis in a pixel-by-pixel, raster-like fashion. Moreover, in microscopy of living systems phototoxicity is a concern, especially in laser scanning microscopy because of the high intensity and focused nature of illumination. In single-photon CLSM a large subset of emitted photons is purposely blocked, only a fraction will pass the pinhole, making the efficiency of use of emission signals very inefficient. Efficient collection of emitted photons is further hampered by the relatively low quantum efficiency of photon detectors. Thus, fluorescence confocal microscopy allows high-resolution 3D fluorescence reconstruction of live specimens, but phototoxicity and photobleaching are of concern and fast dynamic processes can only be imaged by sacrificing spatial resolution or field of view.

To enable faster and more efficient collection of signals for subcellular microscopic analysis widefield, optical sectioning can be achieved by single-plane illumination microscopy (SPIM) [23]. In SPIM, the illumination path is placed perpendicular to the detection



	Widefield	CLSM	MPE CLSM	SPIM
Collection	Full stack	Pixel-by-pixel	Pixel-by-pixel	Plane-by-plane
Acquisition speed	+++	-	-	++
Lateral resolution	+	+++	+++	++
Lateral photodamage	+	+++	+++	++
Axial resolution	-	+++	+++	++
Axial photodamage	+	++	-	+
Zebrafish suitability	+	++	++	+++

Fig. 1. Fluorescence microscopy in zebrafish imaging. Widefield fluorescence microscopy images the whole field-of-view without filtering out-of-focus light, resulting in relatively high acquisition speeds and low photodamage at the cost of lateral and axial resolution. CLSM uses a pinhole for optical sectioning, resulting in relatively high resolution at the cost of increased photo-damage and decreased acquisition speeds, due to the need of pixel-by-pixel scanning of a powerful laser. Axial photodamage can be reduced in MPE where excitation only occurs in the focal plane. In SPIM, the detection light path is perpendicular to the illumination light path allowing increased acquisition speeds and decreased photodamage.

objective. With the complete illuminated plane imaged, excitation is limited to the field-of-view making the excitation/emission efficiency extremely high. Efficient signal collection is further increased through the use of high quantum yield cameras (sCMOS or EM-CCD), which also allow faster acquisition times needed for *in vivo* imaging of dynamic (sub)cellular events. The current generation of commercial SPIM microscopes is nowadays becoming standard equipment in core facilities. In the context of T1D research, the zebrafish pancreas is the tissue of interest in which advanced microscopy allows efficient and fast assessment of dynamic subcellular biological processes.

The zebrafish early larvae pancreas

Zebrafish pancreata have a similar, albeit simpler, morphology when compared to the human pancreas (Fig. 2A). In the early zebrafish larvae one primary islet of Langerhans, 'the principal islet', surrounded by exocrine tissue is formed with secondary islets rarely present before 120 hpf [24]. As in mammals, the zebrafish pancreas plays a role in both the digestion of food through the release of digestive enzymes by exocrine cells and glucose homeostasis through the release of hormones by endocrine cells (Fig. 2B,C). The adult islets contain distinct hormone-producing cells, like alpha cells (glucagon), beta cells (insulin), delta cells (somatostatin) and gamma cells (pancreatic polypeptide) [25].

Development of the pancreas has been studied in detail, and given the limited zebrafish-specific antibodies available, typically the presence of mRNA has been assessed through *in situ* hybridization. Cells positive for pancreatic and duodenal homeobox 1 (PDX1) mRNA are present at the 10-somite stage (14 hpf). Commitment to endocrine fates can be observed at 12-somite stage (15 hpf; insulin), the 16-somite stage (17 hpf; somatostatin) and the 24-somite stage (21 hpf; glucagon) [26]. In contrast, mRNA encoding the digestive enzyme trypsin has only been detected between 48 hpf and 72 hpf in the same cell cluster, leading early studies to conclude the zebrafish pancreas develops from a single progenitor structure [27]. Subsequent studies revealed that, like in mammals, the zebrafish pancreas develops from two pancreatic progenitor structures: the dorsal and the ventral bud [28]. The ventral bud appears after 40 hpf and its fusion with the dorsal bud is complete at 52 hpf which coincides with the earlier observation of trypsin mRNA expression. The dorsal bud is the cell cluster that contributes solely to the formation of the principal islet while the ventral bud appears to give rise to all pancreatic cell types. Interestingly, the dorsal bud-derived beta cells appear to be quiescent after differentiation while additional endocrine cells are derived from ventral

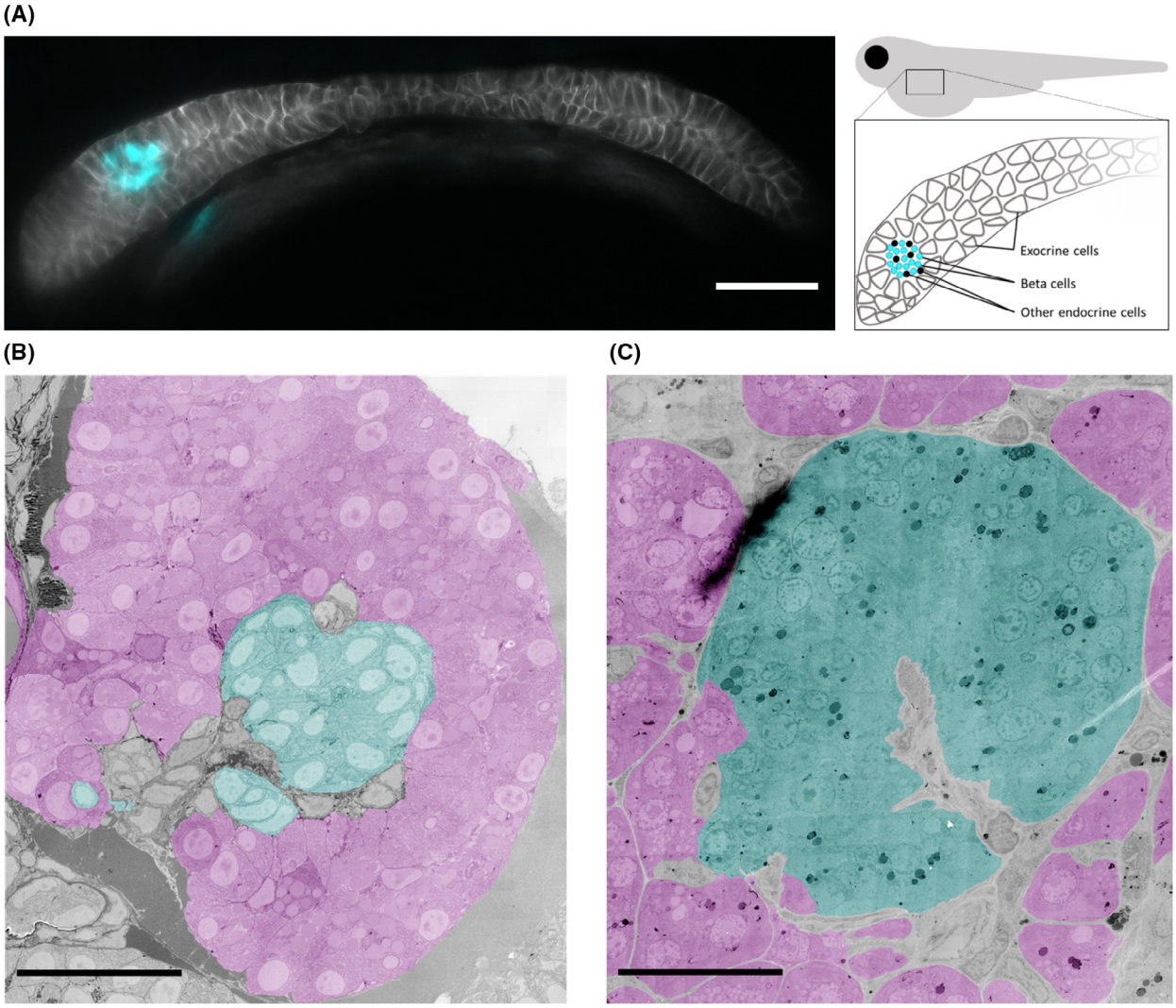


Fig. 2. Zebrafish pancreas morphology. (A) Sagittal plane imaging demonstrates pancreatic morphology in a 120 hpf zebrafish expressing GFP in beta cells (cyan) and membrane-localized mScarlet in the exocrine pancreas (white). Images were acquired on a SPIM microscope of membrane-localized mScarlet under control of the elastase A promoter and cytoplasmic GFP under the insulin promoter. Schematic representation of the head of the zebrafish pancreas is given on the right. (B) Large-scale electron micrograph ('Nanotomy') of the islet of Langerhans surrounded by exocrine tissue in zebrafish and (C) humans. Overlays indicate the endocrine cells (cyan) and acinar cells (magenta) as identified based on ultrastructure. Zoomable images are available via nanotomy.org. Bars: (A) 50 μm, (B and C) 25 μm.

bud-derived cells [24,29]. Taken together, differentiated pancreatic cells make up a premature pancreas in the developing early zebrafish larva making it a powerful model of pancreas (patho)physiology. While we aim to understand the aetiology of T1D, further details of the developing pancreas are already available in an excellent collection of literature [30,31].

Modulating islet function in zebrafish

The comparable pancreas structure and function, zebrafish features and live cell microscopy techniques form a unique triad that allows the study of T1D onset and development. To modulate islet function in zebrafish, several approaches have been developed and are being used (Fig. 3). Ablation approaches are applied to zebrafish attempting to directly imitate the effect of beta cell absence and thus full-blown T1D. This can be done not only by surgical removal of the islet but also using photodamage, chemical and genetic-based modelling approaches or combinations thereof. As opposed to direct ablation, more subtle cell dysfunction techniques that gradually promote beta cell failure are used to get more insight into the onset of T1D. The different

approaches, as well as the benefits and drawbacks of each method, are outlined below.

Surgical removal of islets

Pancreatectomy is the surgical removal of the pancreas which was the key experiment to link pancreas to T1D as outlined above. Islet-targeted fluorescence in adult transgenic zebrafish can be used as guidance together with forceps to excise the FP-positive islets in zebrafish (Fig. 3A). Interestingly, if the islet is not completely removed, the surviving beta cells appear to regenerate, proving it to be a useful tool in islet regeneration studies in adult zebrafish [3]. One downside is that not only the beta cells but also other endocrine cells are removed.

Photodamage-induced ablation

Multi-photon excitation can be utilized to deliver a damaging high dose of photons to a specific area (Fig. 3B). Target cells are typically identified by tissue-specific expression of an FP, where the focal nature allows minimal off-target effects [32,33]. Two-photon excitation has previously successfully been used to ablate beta cells and explore the heterogeneity of beta cell function in the zebrafish islet [34,35]. The key advantages of photo destruction are the precision and speed with which individual or groups of cells can be damaged, but there are alternatives that are better suited to ablate larger areas.

Chemical-induced destruction

Chemical ablation damages a subset of beta cells or the entire beta cell population in a dose-dependent manner (Fig. 3C). The hydrophilic chemicals alloxan and streptozotocin (STZ) enter via the glucose transporter 2, which is abundantly and uniquely expressed in the beta cell membrane [36]. Subsequently, alloxan and STZ generate reactive oxygen species and trigger apoptosis or necrosis depending on their concentrations, leading to beta cell destruction [37,38].

Alloxan has a glucose-like structure that produces free radicals and inhibits glucokinase in zebrafish beta cells. Glucokinase functions as a glucose sensor in beta cells and its suppression results in beta cell damage [36]. The drawbacks of alloxan administration in animals include a short duration of hyperglycaemia as well as a high mortality rate due to the generation of high ketoacidosis levels (reviewed in ref. [39]).

Streptozotocin in sub-molar concentrations increases blood glucose levels and reduces the number of beta cells in adult zebrafish [3]. Also, STZ-injected zebrafish larvae demonstrate notably high blood glucose levels as well as extremely low insulin mRNA and protein levels [40]. Therefore, STZ ablation is used to model T1D in zebrafish, as well as in other animals, by fully destroying beta cells and impairing glucose metabolism. This T1D model was utilized to evaluate the efficacy of potential antidiabetic drugs, making use of the high content screening potential of zebrafish larvae that is absent in other vertebrate model systems [40,41]. Overall, STZ is recommended over alloxan for inducing T1D zebrafish due to its higher potency and lower toxicity. Nonetheless, the reported chemical toxicity to other organs is still the main disadvantage of chemical-induced destruction.

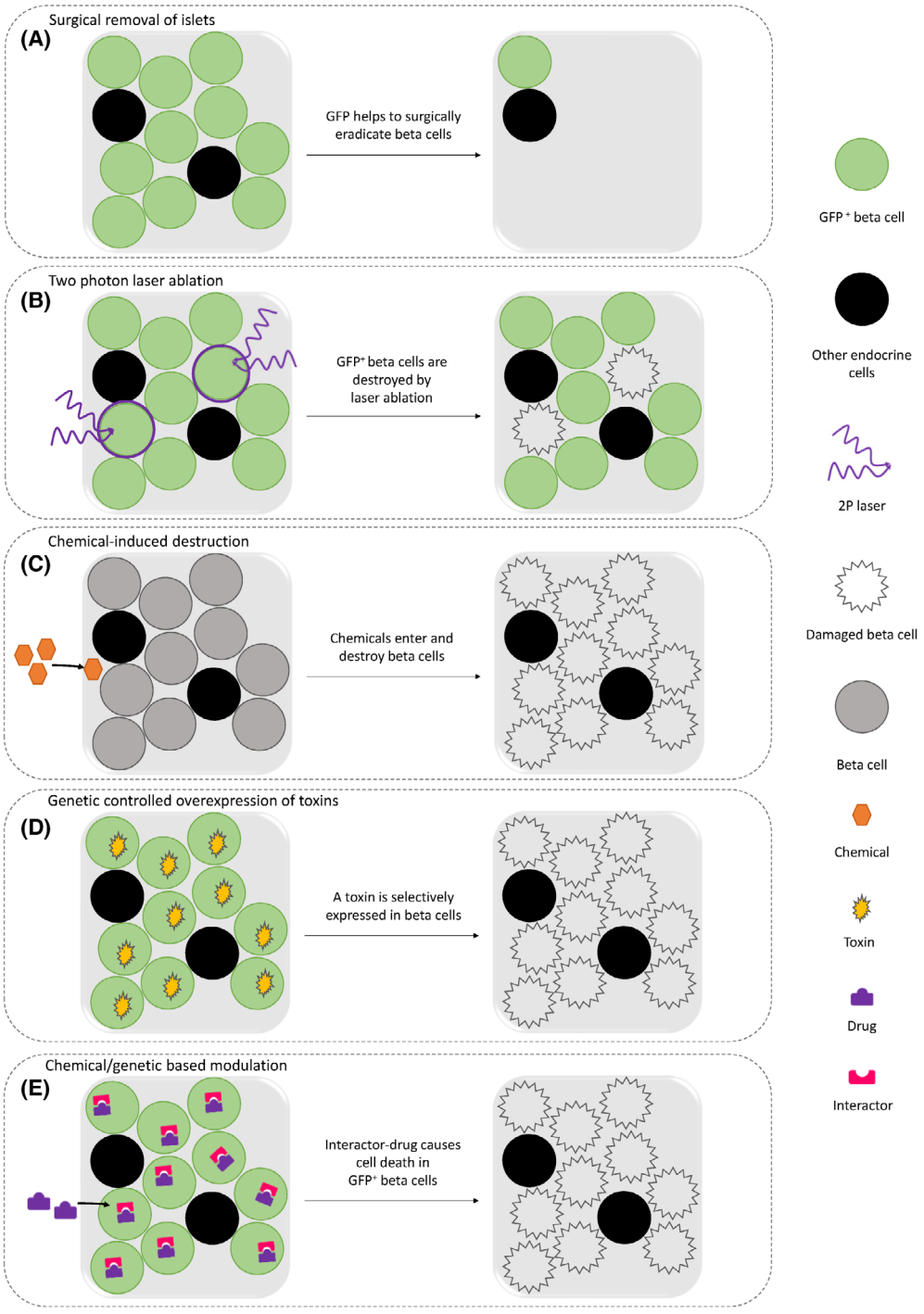
Tissue-specific overexpression of toxins

Expression of toxins can be used to accomplish ablation combined with tissue-specific promoters to ensure cell selectivity (Fig. 3D). Diphtheria toxin A chain (DTA) suppresses cell growth and induces programmed cell death in transgenic mice and zebrafish [42–44]. Selective beta cell damage has been induced through insulin promoter-restricted expression of DTA, allowing Li et al. [45] to investigate the developmental relationship between distinct endocrine cells. However, due to the high toxicity of DTA, the presence of unwanted off-target effects hampers the application of DTA as an ablation strategy [46].

Chemical/genetic modulation

Combining the tissue-restricted genetic-based ablation with the temporal inducibility of the chemical-based method can limit the downsides of both individual methods. Here, genetic modification allows the introduction of tissue-specific interactors such as enzymes, proteins or specific DNA sequences that interact with exogenously supplied chemicals (Fig. 3E). This hybrid approach allows conditional inducibility and has previously been used in animal models where target cells were made susceptible to DTA uptake through expression of the diphtheria toxin receptor [46], off-target effects have been reduced by inducible expression of the receptor using Cre-Lox recombination [47].

The Tet-ON binary transgene expression system is another example of chemical/genetic modulation. Here, the tissue-restricted expressed reverse tetracycline-controlled trans-activator is only activated in the presence of tetracycline, or its analogues, thereby inducing the expression of genes downstream of the tet operator [48]. For beta cell differentiation studies, the Tet-ON system was applied to selectively ablate zebrafish beta



	Surgery	2P laser	Chemical	Genetic	Hybrid
Zebrafish suitability	Adult	Larvae	Adult/larvae	Larvae	Larvae
Inducibility	+	+	+	-	+++
Cell specificity	++	+++	+++	++	+++
Simplicity	+	++	+++	++	++
Damage induction	Minutes	Seconds	Hours	Hours	Hours

Fig. 3. Beta cell modulation in zebrafish. (A) GFP-positive (GFP^+) beta cells can be removed from adult zebrafish using a surgical method. Note that this procedure essentially removes the islet including other endocrine cells. Moreover, given the technique, the balance between attempting to remove the complete islet but not exocrine tissue may leave a few islets cells. (B) With high excitation intensity, laser-based ablation is a flexible tool for damaging targeted individual cells or groups of GFP^+ beta cells. (C) Beta cells are destroyed via uptake of chemical glucose analogues or (D) the expression of toxins. The chemical and genetic procedures, however, are limited by the toxicity associated with chemicals and toxins. (E) The chemical/genetic-based modulation combined. Tissue-restricted genetic ablation and the temporal inducibility of chemical-based methods is indicated. This hybrid approach can mitigate the drawbacks of the individual single procedures. See text for further details.

cells by activating a truncated pro-apoptotic gene in the presence of doxycycline and tebufenozide. This method has previously been used to indicate that existing beta cells are essential for compensating beta cell development following overnutrition [49].

Modulation of apoptosis also can be achieved through synthetic compounds, like AP20187, that induce dimerization of artificial FK506-binding proteins (FKBP) and subsequent activation, initiating apoptosis [50,51]. In 3–5-day post-fertilization zebrafish larvae, caspase 8-FKBP fusion proteins are expressed under tissue-specific promoters such as the insulin and elastase A promoters in beta cells or exocrine cells respectively. In response to AP20187, cells are eliminated within 48 h and allowed in this case to study alpha cell to beta cell transdifferentiation and exocrine neogenesis [52,53].

Nitroaromatic antibiotics are widely used as prodrugs in chemical/genetic ablation in zebrafish. For instance, metronidazole (MTZ) and nifurpirinol are transformed via the nitroreductase enzyme (NTR) into a cytotoxic DNA cross-linking agent, leading to cell death. The MTZ-NTR system has been widely used for conditional beta cell ablation in transgenic zebrafish [54,55]. Nevertheless, high doses and long exposure causes variability in cell ablation effectiveness depending on the cell type [56–58]. To minimize MTZ toxicity, a triple mutant NTR was developed to reduce MTZ concentration and/or exposure time [59]. However, even with the mutant version, microbiome interference and inefficient cell damage are observed at long exposure times and maximum dose [52]. Therefore, similar nitroaromatic antibiotics were assessed to investigate their potency in causing similar damage at lower doses [60,61]. For instance, nifurpirinol is being evaluated in a variety of cell types, including beta cells. This prodrug has been found to be a more potent MTZ alternative because nifurpirinol causes partial or complete cell damage at lower concentrations and has less general toxicity than MTZ [60].

The ganciclovir-HSV-TK system uses a similar approach where the thymidine kinase (TK) of the herpes simplex virus (HSV) converts the prodrug ganciclovir into a toxic reagent, destroying TK-positive cells. For studying beta cell proliferation, zebrafish embryos were treated with ganciclovir, resulting in the ablation of all beta cells expressing TK under the zebrafish insulin promoter [62,63]. All in all, chemical/genetic-based modulation methods are most widely used in pancreatic research and the development of T1D models.

Stressors

The functionality of beta cells can also be hampered more subtly as opposed to complete ablation, including in several of the systems above by adjusting exposure time, concentration or intensity to the modulating agent. Impaired functionality has been induced in zebrafish by expressing the pro-inflammatory cytokine interleukin-1 beta under the insulin promoter, resulting in inflammation and immune cell recruitment. As a result, an abolished glucose-stimulated calcium response and lower expression of critical beta cell genes was observed [64]. An expansion of this model was recently introduced where the Cre-dependent and tetracycline-inducible expression of equimolar concentrations of inflammatory cytokines was used to trigger beta cell stress [65]. Systemic administration of monovalent 1 initiates inflammation by activating NF-κB inducing kinase and creating a glucose imbalance [66]. Targeting key molecules involved in insulin secretion, like the ATP-sensitive potassium channel that is indirectly activated by glucose, can also be used to modulate beta cell function as demonstrated previously [67]. These models can thus be used to more subtile assess the effect of beta cell dysfunction on pancreas physiology in living zebrafish larvae.

Optogenetics

Exogenous light-sensitive proteins can modulate cellular function or induce cell death. A variety of chromophores have been engineered to drive manipulation of specific cell signalling pathways and multiple of these optogenetic tools have previously been applied in

zebrafish. While their application in the zebrafish pancreas has remained limited, we foresee a broad implementation of these tools to better understand islet function in the years to come. The photosensitizer KillerRed has previously been used to induce apoptosis in neuronal and cardiac tissue of larval zebrafish [68]. More recently, a sophisticated approach to inducing neuronal cell death through light-controlled transcription of a cytotoxic ion channel was implemented in zebrafish [69]. Light-controlled transcription has also previously been used to induce insulin translation and expression *in vivo* by implanting engineered cells in mice [70]. Additionally, multiple optogenetic tools have been developed to control beta cell activity both *in vitro* and *in vivo*. Channelrhodopsin-2 (ChR2) is a light-responsive cation channel that has been successfully used to depolarize beta cell-like cells and beta cells in mice, leading to Ca^{2+} influx and subsequent insulin release [71,72]. In contrast, beta cells have been successfully silenced through hyperpolarization in mouse islets using the light-responsive chlorine pump halorhodopsin [73]. Furthermore Ca^{2+} signalling can be controlled more directly through the light-controlled activation of calcium release-activated channels as used in the OptoSTIM1 and Opto-CRAC systems [74,75]. Optogenetics offers high spatial-temporal control over ablation and modulation strategies and has already proven to be a valuable tool in studies of beta cell function in other organisms or tissues. The combination of microscopic techniques (Fig. 1), the zebrafish larvae and optogenetic tools have great potential to modulate and analyse islet function *in vivo*.

Overall, successful applications of a variety of modulation techniques have assisted in the study of pancreatic development and (patho)physiology, albeit each having pros and cons depending on the question at issue (Fig. 3). The next step is *in vivo* analysis of islet physiology following the *in vivo* modulation approaches. Transgenic reporter lines are continuously created to facilitate studies on pancreatic development and function in zebrafish. Pairing reporter lines with advanced microscopy techniques allows the visualization of subcellular dynamic events in living zebrafish. Therefore, the corresponding application can aid in investigating cellular dysfunction or abnormalities in a zebrafish T1D model.

In vivo subcellular readouts of pancreas physiology

Cell organelles and signalling pathways can be used to spy on pancreas (patho)physiology (Table 1). Calcium release from the endoplasmic reticulum (ER) is required to initiate insulin excretion in beta cells and real-time monitoring of cytoplasmic beta cell calcium dynamics is therefore a key indicator of beta cell function [76,77]. Moreover, mitochondrial and ER stress induces oxidative stress and apoptosis, causing beta cell malfunction [78,79]. Therefore, the status of these organelles provides prominent indicators of beta cell performance. Additionally, sensors of inflammation can mark insulitis. In beta cells, these various processes are interwoven. Below, we highlight multiple fluorescent biosensors that report beta cell presence, calcium flux, ER status, mitochondria function and

Table 1. Readouts of pancreatic cells in transgenic zebrafish. Endocrine (rows provided in italic font) and exocrine (rows provided in bold italic font) cells have distinct functions, yet they exist in the same pancreatic structure and share a common pdx1+ progenitor [111,112]. Therefore, fluorescent reporter lines are used to identify the distinguished transitions of pancreatic development in the zebrafish model. Both endocrine and exocrine reporter lines were thoroughly reviewed previously [113,114].

Indicator	Reporter and/or readout	References
Ca^{2+} dynamics	ins:Rcamp	[83]
	ins:GCaMP6s;cryaa:RFP	[35]
Beta cell mass	ptf1a:GFP/insa:mCherry	[86]
ER stress	XBP1Δ-GFP	[92]
	5XATF6RE:eGFP	[93]
Mitochondria stress	Grx1-roGFP2	[115]
	roGFP2-Orp1	[115]
	HyPer-3	[116]
Apoptosis	ZipGFP	[102]
	FlipGFP	[104]
	SecA5-YFP	[103]
Inflammation	mpeg1:mCherry	[97]
	NF-κB:EGFP	[96]
Metabolite state	iGlucSnFR	[106]
	iNap	[100]
	SoNar	[107]
	FiNad	[108]
	PercevalHR	[109]
Development	nkx2.2a:GFP	[117]
	ptf1a:eGFP	[127]
	ptf1a:eGFP-KRAS G12V	
	hb9:GFP	[118]
	mnx1:GFP	[119]
	ascl1b:eGFP-2A-creERT2	[120]
	nkx6.1:eGFP	[120]
	neurod:egfp	[121]
	pax6b:gfp	[122]
Progenitors and ductal cells	6.5pdx1:GFP	[123]
Alpha cells	gcga:GFP	[124]
Delta cells	sst2:eGFP	[25]
Beta cells	ins:dsRed	[125]
Acinar cells	elaA:gfp	[126]

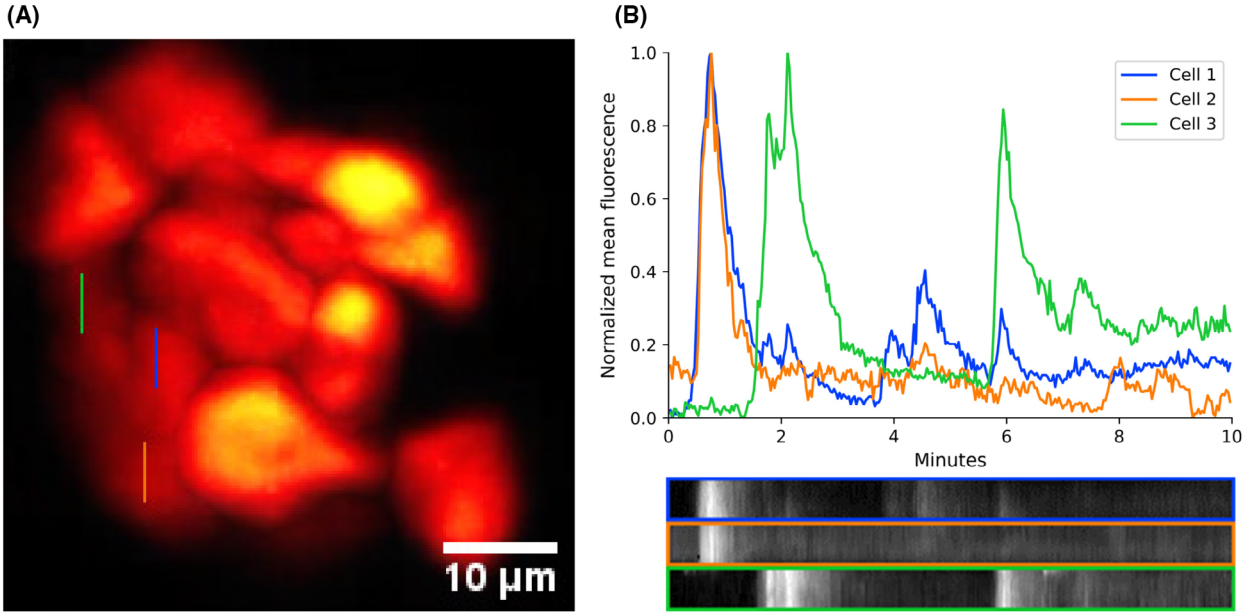


Fig. 4. *In vivo* physiological readouts with high spatiotemporal resolution. Zebrafish expressing the calcium indicator Rcamp1.07 under the insulin promoter were stimulated with glucose and volumetric time lapse imaging was conducted using SPIM by Zhao et al. [83]. Maximum intensity projection in time indicates the cells selected for measurement (A). Calcium dynamics can be visualized by plotting the normalized fluorescence with an accompanying kymograph showing the normalized regions of interest over time (B). Data of an image sequence were published under a CC-BY 4.0 license in eLife and reused and repurposed [83]. DOI: 10.7554/eLife.41540.010.

inflammatory state at a subcellular level in living zebrafish.

Ca^{2+} dynamics

Genetically encoded Ca^{2+} indicators have been successfully evolved into powerful tools to assess calcium dynamics in beta cells of living zebrafish at the single-cell resolution [80]. GCaMPs are such artificially developed indicators, and consist of a multidomain protein that includes GFP, calmodulin and the M13 segment of myosin light chain kinase. GCaMPs do not fluoresce in the absence of calcium, while high intracellular calcium levels cause a conformational change in GCaMPs by M13 leading to GFP fluorescence [81]. GCaMP expression under a tissue-specific promoter allows monitoring of calcium flux in those cells, which was used to study *in vivo* beta cell development and function in zebrafish [34,35,82]. New variants of genetically encoded indicators have been applied to visualize Ca^{2+} dynamics in beta cells in 3D using advanced microscope technologies such as SPIM (Fig. 4) [83–85].

Beta cell mass

Fluorescent markers allow individual beta cells to be counted in zebrafish larvae to assess the loss of beta cells, as seen in patients with T1D. To account for developmental changes, the number of beta cells can be interpreted as a ratio to the number of exocrine cells. This ratio does not reflect individual beta cell performance, but might be a good prediction of total insulin secretion potency of the islets of Langerhans [86].

ER homeostasis

The ER network is abundant in beta cells and involved in insulin biosynthesis, folding, trafficking and exocytosis. Under stressful conditions, misfolded proteins accumulate and cause ER stress by inducing the unfolded protein response [87,88], a major hallmark of early beta cell damage in T1D [89,90]. A central modulator of the unfolded protein response is alternative splicing of X-box binding protein-1 mRNA [91]. Optical fluorescent sensors to report on splicing of X-box binding protein-1 mRNA have been developed and applied in zebrafish [92]. ER stress also leads to activation of Activating Transcription Factor 6 (ATF6), which too can be monitored using optical sensors [93]. Importantly, ER stress may lead to neo-epitope formation [94], which may be an essential step in triggering the immune system to specifically start to attack the beta cells. Therefore, ER function can be

utilized as a powerful readout of beta cell functionality.

Inflammation

Endoplasmic reticulum stress and unfolded protein response activation also trigger an inflammatory response, resulting in cytokine release and beta cell death [95]. Activation of NF-kB can be used to read out the inflammatory response of cells to stimuli [96]. Moreover, migration and/or infiltration of immune cells can be tracked using appropriate promoters such as the mpeg1 promoter for macrophages [97]. Thus, inflammatory responses and their outcomes can be visualized and tracked dynamically using live cell imaging of the larval zebrafish pancreas.

Mitochondrial function

Mitochondria are essential to couple glucose metabolism to insulin secretion under normal physiological conditions and play a major role in apoptotic beta cell death [98]. A large panel of different genetically encoded thiol redox sensors, which are available in zebrafish, report the oxidative stress status in mitochondria in real time *in vivo* [99–101]. Since the oxidative stress may also trigger apoptosis, apoptotic sensors are available in zebrafish. For instance, zipGFP and FlipGFP can be used to report caspase cleavage and annexinV fused to a FP can be used to visualize phosphatidylserine on the outer membrane [102–104]. Therefore, mitochondrial (dys)function can be assessed in the context of beta cell stress.

Metabolite sensors

T1D is linked to a metabolic imbalance, leading to alterations in glucose, ATP, and NADH levels [105]. In normal metabolism, glucose can facilitate generation of pyruvate, ATP and NADH. Many genetically based fluorescence sensors are able to track the metabolite state in zebrafish studies, yet not in pancreas research. For instance, the intensity-based reporter iGlucoSnFR, allows monitoring intracellular glucose in zebrafish muscle, liver and brain in real time [106]. Other metabolic sensors such as iNap, SoNar, FiNad and PercevalHR dynamically track NADPH fluctuations, NAD^+/NADH ratio, NAD^+ metabolic change and ATP/ADP ratio, respectively, in live zebrafish larvae [100,107–109].

Various genetically modified fluorescent sensors facilitate tracking biochemical changes in zebrafish such as redox status, metabolites, messengers and stress reporters, as well as cellular events such as apoptotic cell death. These sensors can facilitate imaging of dynamic events in the zebrafish pancreas through microscopy and thus give insights into the biochemical status of beta cells.

Future outlook

Microscopy is a powerful tool to dynamically visualize biological processes and, with the discovery and development of fluorescent proteins, live-cell imaging in cellular systems has exponentially expanded. A drawback of imaging larger volumes at subcellular resolution is the relatively long time required to acquire the data, especially when using pixel-by-pixel scanning. Moreover, *in vivo* imaging at high spatiotemporal resolution while limiting phototoxicity remains a challenge. Efficient excitation and collection of emission in light-sheet microscopy (SPIM) and decades of development of the SPIM technique have now reached the level of stable, user-friendly machines, and typically also sample holders on SPIM microscopes have been adapted to analyse the small organisms. These advances in technology allow scientists to visualize previously unexplored avenues of research to further expand our understanding of dynamics in biology.

Zebrafish are small vertebrate animal models that have great potential in overcoming the current drawbacks of common models used in biomedical research. Many animal models overcome the lack of multicellular context that is present in *in vitro* studies but are often complex to work with and are not suitable for real-time analysis. Due to their small size, transparency and ease of genetic engineering zebrafish larvae make great models to conduct intravital imaging. For T1D research, the pancreas is of great interest and we are still happily amazed at how fast the pancreas nicely develops into a functional organ within only a few days after fertilization. Likewise, other researchers have appreciated the supplementary value of zebrafish, as evidenced by their use in studies on pancreas development [27,28], beta cell regeneration in zebrafish [93] and studying secondary complications associated with elevated blood glucose levels [94]. Recent insights reveal that T1D is a disease not only of the beta cells but also of the pancreas as a whole [95], which can be perfectly studied in the larvae with the help of the latest molecular tools.

With the implementation of GFP, fluorescent proteins stood at the core of the revolution in fluorescence microscopy. Ever since, fluorescent probes have allowed us to follow the fate of proteins and processes

of interest in space and time. Indeed, fluorescent proteins have been modified and fused to other proteins not only to follow them more efficiently but also to dynamically reflect the biochemical status of cells. Many mechanisms have been uncovered and exploited to flexibly modulate cellular function and fate, and to investigate the consequences of these modulations. Furthermore, optogenetic tools are increasingly being developed and exploited to obtain an even more precise spatiotemporal control. The accompanying additional flexibility in affecting cellular function and fate shows great promise for generic use in biomedical research using model systems such as the zebrafish.

The fluorescent toolbox, zebrafish technologies and revolutionary non-invasive microscopy methods such as SPIM have now converged and allow researchers to functionally interfere with cells, as well as to study biology with sub-cellular resolution in the native *in situ* context. Here, we emphasized how this combination can be used to mimic T1D progression and visualize the effects of the loss of (functional) beta cells to further complement current T1D research approaches. Surely, this unique triad will help advance studies in the field of T1D and beyond.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

NF, BHPD and BNGG drafted the first version and created Figures and Table. All authors edited the manuscript.

References

1 Langerhans P. Beiträge zur mikroskopischen Anatomie der Bauchspeicheldrüse. Berlin: Buchdruckerei von Gustav Lange; 1869.
2 von Mering J, Minkowski O. Diabetes mellitus nach Pankreasexstirpation. *Arch Exp Pathol Pharmakol*. 1890;**26**:371–87.
3 Moss JB, Koustubhan P, Greenman M, Parsons MJ, Walter I, Moss LG. Regeneration of the pancreas in adult zebrafish. *Diabetes*. 2009;**58**:1844–51.
4 Banting FG, Best CHJBC, Campbell WR, Fletcher AA, MacLeod JJR, Noble EC. The effect produced on diabetes by extracts of pancreas. *Trans Assoc Am Physicians*. 1922;**37**:337–47.
5 Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic extracts in the treatment of diabetes mellitus. *Can Med Assoc J*. 1922;**12**:141–6.
6 Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes*. 1965;**14**:619–33.
7 Gepts W, De Mey J. Islet cell survival determined by morphology. An immunocytochemical study of the islets of Langerhans in juvenile diabetes mellitus. *Diabetes*. 1978;**27**:251–61.
8 de Boer P, Giepmans BNG. State-of-the-art microscopy to understand islets of Langerhans: what to expect next? *Immunol Cell Biol*. 2021;**99**:509–20.
9 King AJ. The use of animal models in diabetes research. *Br J Pharmacol*. 2012;**166**:877–94.
10 Zhang X, Ma Z, Song E, Xu T. Islet organoid as a promising model for diabetes. *Protein Cell*. 2022;**13**:239–57.
11 Joshi K, Cameron F, Tiwari S, Mannering SI, Elefanty AG, Stanley EG. Modeling type 1 diabetes using pluripotent stem cell technology. *Front Endocrinol (Lausanne)*. 2021;**12**:635662.
12 Dooley K, Zon LI. Zebrafish: a model system for the study of human disease. *Curr Opin Genet Dev*. 2000;**10**:252–6.
13 Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al. The zebrafish reference genome sequence and its relationship to the human genome. *Nature*. 2013;**496**:498–503.
14 Strähle U, Scholz S, Geisler R, Greiner P, Hollert H, Rastegar S, et al. Zebrafish embryos as an alternative

to animal experiments-a commentary on the definition of the onset of protected life stages in animal welfare regulations. *Reprod Toxicol*. 2012;**33**:128–32.
15 Rafferty SA, Quinn TA. A beginner's guide to understanding and implementing the genetic modification of zebrafish. *Prog Biophys Mol Biol*. 2018;**138**:3–19.
16 Kawakami K, Takeda H, Kawakami N, Kobayashi M, Matsuda N, Mishina M. A transposon-mediated gene trap approach identifies developmentally regulated genes in zebrafish. *Dev Cell*. 2004;**7**:133–44.
17 Kawakami K. Tol2: a versatile gene transfer vector in vertebrates. *Genome Biol*. 2007;**8**:1–10.
18 Sertori R, Trengove M, Basheer F, Ward AC, Liongue C. Genome editing in zebrafish: a practical overview. *Brief Funct Genomics*. 2016;**15**:322–30.
19 Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker for gene expression. *Science*. 1994;**263**:802–5.
20 Giepmans BNG, Adams SR, Ellisman MH, Tsien RY. The fluorescent toolbox for assessing protein location and function. *Science*. 2006;**312**:217–24.
21 Sanderson MJ, Smith I, Parker I, Bootman MD. Fluorescence microscopy. *Cold Spring Harb Protoc*. 2014;**2014**:pdb.top071795.
22 Diaspro A, Bianchini P, Vicidomini G, Faretta M, Ramoino P, Usai C. Multi-photon excitation microscopy. *Biomed Eng Online*. 2006;**5**:36.
23 Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer EHK. Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science*. 2004;**305**:1007–9.
24 Parsons MJ, Pisharath H, Yusuff S, Moore JC, Siekmann AF, Lawson N, et al. Notch-responsive cells initiate the secondary transition in larval zebrafish pancreas. *Mech Dev*. 2009;**126**:898–912.
25 Li Z, Wen C, Peng J, Korzh V, Gong Z. Generation of living color transgenic zebrafish to trace somatostatin-expressing cells and endocrine pancreas organization. *Differentiation*. 2009;**77**:128–34.
26 Biemar F, Argenton F, Schmidtke R, Epperlein S, Peers B, Driever W. Pancreas development in zebrafish: early dispersed appearance of endocrine hormone expressing cells and their convergence to form the definitive islet. *Dev Biol*. 2001;**230**:189–203.
27 Argenton F, Zecchin E, Bortolussi M. Early appearance of pancreatic hormone-expressing cells in the zebrafish embryo. *Mech Dev*. 1999;**87**:217–21.
28 Field HA, Si Dong PD, Beis D, Stainier DYR. Formation of the digestive system in zebrafish. II Pancreas morphogenesis. *Dev Biol*. 2003;**261**:197–208.
29 Hesselson D, Anderson RM, Beinat M, Stainier DYR. Distinct populations of quiescent and proliferative pancreatic beta-cells identified by HOTcre mediated labeling. *Proc Natl Acad Sci USA*. 2009;**106**:14896–901.
30 Kimmel RA, Meyer D. Zebrafish pancreas as a model for development and disease. *Methods Cell Biol*. 2016;**134**:431–61.
31 Tiso N, Moro E, Argenton F. Zebrafish pancreas development. *Mol Cell Endocrinol*. 2009;**312**:24–30.
32 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*. 2018;**136**:e57485.
33 König K. Cell damage during multi-photon microscopy. In: Pawley J, editor. Handbook of biological confocal microscopy. Boston, MA: Springer; 2006. p. 680–9.
34 Salem V, Silva LD, Suba K, Georgiadou E, Neda Mousavy Gharavy S, Akhtar N, et al. Leader β-cells coordinate Ca2+ dynamics across pancreatic islets in vivo. *Nat Metab*. 2019;**1**:615–29.
35 Singh SP, Janjuha S, Hartmann T, Kayisoglu Ö, Konantz J, Birke S, et al. Different developmental histories of beta-cells generate functional and proliferative heterogeneity during islet growth. *Nat Commun*. 2017;**8**:1–15.
36 Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*. 2008;**51**:216–26.
37 Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*. 2001;**50**:537–46.
38 AMTA N, John A, Raza H. Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in Rin-5F pancreatic β -cells. *Oxid Med Cell Longev*. 2017;**2017**:1–15.
39 Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina (B Aires)*. 2017;**53**:365–74.
40 Wang X, Yang X, Liu K, Sheng W, Xia Q, Wang R, et al. Effects of streptozotocin on pancreatic islet β-cell apoptosis and glucose metabolism in zebrafish larvae. *Fish Physiol Biochem*. 2020;**46**:1025–38.
41 Benchoula K, Khatib A, Quzwain FMC, Che Mohamad CA, Wan Sulaiman WMA, Abdul Wahab R, et al. Optimization of hyperglycemic induction in zebrafish and evaluation of its blood glucose level and metabolite fingerprint treated with Psychotria malayana Jack leaf extract. *Molecules*. 2019;**24**:1506.
42 Palmiter RD, Behringer RR, Quaife CJ, Maxwell F, Maxwell IH, Brinster RL. Cell lineage ablation in transgenic mice by cell-specific expression of a toxin gene. *Cell*. 1987;**50**:435–43.

43 Breitman ML, Clapoff S, Rossant J, Tsui L-C, Glode LM, Maxwell IH, et al. Genetic ablation: targeted expression of a toxin gene causes microphthalmia in transgenic mice. *Science*. 1987;**238**:1563–5.

44 Kurita R, Sagara H, Aoki Y, Link BA, Arai K, Watanabe S. Suppression of lens growth by αA-crystallin promoter-driven expression of diphtheria toxin results in disruption of retinal cell organization in zebrafish. *Dev Biol*. 2003;**255**:113–27.

45 Li Z, Korzh V, Gong Z. DTA-mediated targeted ablation revealed differential interdependence of endocrine cell lineages in early development of zebrafish pancreas. *Differentiation*. 2009;**78**:241–52.

46 Saito M, Iwawaki T, Taya C, Yonekawa H, Noda M, Inui Y, et al. Diphtheria toxin receptor–mediated conditional and targeted cell ablation in transgenic mice. *Nat Biotechnol*. 2001;**19**:746–50.

47 Buch T, Heppner FL, Tertilt C, Heinen TJAJ, Kremer M, Wunderlich FT, et al. A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat Methods*. 2005;**2**:419–26.

48 Buchholz DR. Tet-on binary systems for tissue-specific and inducible transgene expression. *Methods Mol Biol*. 2012;**917**:265–75.

49 Li M, Maddison LA, Page-McCaw P, Chen W. Overnutrition induces β-cell differentiation through prolonged activation of β-cells in zebrafish larvae. *Am J Physiol Endocrinol Metab*. 2014;**306**:799–807.

50 Carlotti F, Zaldumbide A, Martin P, Boulukos KE, Hoeben RC, Pognonec P. Development of an inducible suicide gene system based on human caspase 8. *Cancer Gene Ther*. 2005;**12**:627–39.

51 Oberst A, Pop C, Tremblay AG, Blais V, Denault J-B, Salvesen GS, et al. Inducible dimerization and inducible cleavage reveal a requirement for both processes in Caspase-8 activation. *J Biol Chem*. 2010;**285**:16632–42.

52 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*. 2017;**10**:307–21.

53 Li J, Casteels T, Frogne T, Ingvorsen C, Honoré C, Courtney M, et al. Artemisinins target GABA a receptor signaling and impair α cell identity. *Cell*. 2017;**168**:86–100.e15.

54 Pisharath H, Parsons MJ. Nitroreductase-mediated cell ablation in transgenic zebrafish embryos. *Methods Mol Biol*. 2009;**546**:133–43.

55 Curado S, Stainier DYR, Anderson RM. Nitroreductase-mediated cell/tissue ablation in zebrafish: a spatially and temporally controlled ablation method with applications in developmental and regeneration studies. *Nat Protoc*. 2008;**3**:948–54.

56 Hsu C-C, Hou M-F, Hong J-R, Wu J-L, Her GM. Inducible male infertility by targeted cell ablation in zebrafish testis. *Marine Biotechnol*. 2010;**12**:466–78.

57 Kulkarni AA, Conteh AM, Sorrell CA, Mirmira A, Tersey SA, Mirmira RG, et al. An in vivo zebrafish model for interrogating ROS-mediated pancreatic β-cell injury, response, and prevention. *Oxid Med Cell Longev*. 2018;**2018**:1324739.

58 Lambert AM, Bonkowsky JL, Masino MA. The conserved dopaminergic Diencephalospinal tract mediates vertebrate locomotor development in zebrafish larvae. *J Neurosci*. 2012;**32**:13488–500.

59 Mathias JR, Zhang Z, Saxena MT, Mumm JS. Enhanced cell-specific ablation in zebrafish using a triple mutant of Escherichia Coli Nitroreductase. *Zebrafish*. 2014;**11**:85–97.

60 Bergemann D, Massoz L, Bourdouxhe J, Carril Pardo CA, Voz ML, Peers B, et al. Nifurpirinol: a more potent and reliable substrate compared to metronidazole for nitroreductase-mediated cell ablations. *Wound Repair Regen*. 2018;**26**:238–44.

61 Lai S, Kumari A, Liu J, Zhang Y, Zhang W, Yen K, et al. Chemical screening reveals Ronidazole is a superior prodrug to metronidazole for nitroreductase-induced cell ablation system in zebrafish larvae. *J Genet Genomics*. 2021;**48**:1081–90.

62 Beck C, Cayeux S, Lupton SD, Dörken B, Blankenstein T. The thymidine kinase/ganciclovir-mediated 'suicide' effect is variable in different tumor cells. *Hum Gene Ther*. 1995;**6**:1525–30.

63 Moro E, Gnügge L, Braghetta P, Bortolussi M, Argenton F. Analysis of beta cell proliferation dynamics in zebrafish. *Dev Biol*. 2009;**332**:299–308.

64 Delgadillo-Silva LF, Tsakmaki A, Akhtar N, Franklin ZJ, Konantz J, Bewick GA, et al. Modelling pancreatic β-cell inflammation in zebrafish identifies the natural product wedelolactone for human islet protection. *Dis Model Mech*. 2019;**12**:dmm036004.

65 Ibrahim S, Harris-Kawano A, Haider I, Mirmira RG, Sims EK, Anderson RM. A novel Cre-enabled tetracycline-inducible transgenic system for tissue-specific cytokine expression in the zebrafish: CETI-PIC3. *Dis Model Mech*. 2020;**13**:dmm042556.

66 Malle EK, Zammit NW, Walters SN, Koay YC, Wu J, Tan BM, et al. Nuclear factor κB-inducing kinase activation as a mechanism of pancreatic β cell failure in obesity. *J Exp Med*. 2015;**212**:1239–54.

67 Emfinger CH, Lőrincz R, Wang Y, York NW, Singareddy SS, Ikle JM, et al. Beta-cell excitability and excitability-driven diabetes in adult zebrafish islets. *Physiol Rep*. 2019;**7**:e14101.

68 Teh C, Chudakov DM, Poon K-L, Mamedov IZ, Sek J-Y, Shidlovsky K, et al. Optogenetic in vivocell manipulation in KillerRed-expressing zebrafish transgenics. *BMC Dev Biol*. 2010;**10**:110.

69 Mruk K, Ciepla P, Piza PA, Alnaqib MA, Chen JK. Targeted cell ablation in zebrafish using optogenetic transcriptional control. *Development*. 2020;**147**: dev183640.
70 Yu G, Zhang M, Gao L, Zhou Y, Qiao L, Yin J, et al. Far-red light-activated human islet-like designer cells enable sustained fine-tuned secretion of insulin for glucose control. *Mol Ther*. 2022;**30**:341–54.
71 Reinbothe TM, Safi F, Axelsson AS, Mollet IG, Rosengren AH. Optogenetic control of insulin secretion in intact pancreatic islets with β-cell-specific expression of Channelrhodopsin-2. *Islets*. 2014;**6**: e28095.
72 Kushibiki T, Okawa S, Hirasawa T, Ishihara M. Optogenetic control of insulin secretion by pancreatic β-cells in vitro and in vivo. *Gene Ther*. 2015;**22**:553–9.
73 Johnston NR, Mitchell RK, Haythorne E, Pessoa MP, Semplici F, Ferrer J, et al. Beta cell hubs dictate pancreatic islet responses to glucose. *Cell Metab*. 2016;**24**:389–401.
74 Kyung T, Lee S, Kim JE, Cho T, Park H, Jeong Y-M, et al. Optogenetic control of endogenous Ca2+ channels in vivo. *Nat Biotechnol*. 2015;**33**:1092–6.
75 He L, Zhang Y, Ma G, Tan P, Li Z, Zang S, et al. Near-infrared photoactivatable control of Ca2+ signaling and optogenetic immunomodulation. *Elife*. 2015;**4**:e10024.
76 Lorincz R, Emfinger CH, Walcher A, Giolai M, Krautgasser C, Remedi MS, et al. In vivo monitoring of intracellular ca 2+ dynamics in the pancreatic β-cells of zebrafish embryos. *Islets*. 2018;**10**:221–38.
77 Pérez Koldenkova V, Nagai T. Genetically encoded Ca2+ indicators: properties and evaluation. *Biochim Biophys Acta Mol Cell Res*. 2013;**1833**:1787–97.
78 Vig S, Lambooij JM, Zaldumbide A, Guigas B. Endoplasmic reticulum-mitochondria crosstalk and Beta-cell destruction in type 1 diabetes. *Front Immunol*. 2021;**12**:669492.
79 Eguchi N, Vaziri ND, Dafoe DC, Ichii H. The role of oxidative stress in pancreatic β cell dysfunction in diabetes. *Int J Mol Sci*. 2021;**22**:1509.
80 Li ES, Saha MS. Optimizing calcium detection methods in animal systems: a sandbox for synthetic biology. *Biomolecules*. 2021;**11**:343.
81 Nakai J, Ohkura M, Imoto K. A high signal-to-noise Ca2+ probe composed of a single green fluorescent protein. *Nat Biotechnol*. 2001;**19**:137–41.
82 Janjuha S, Pal Singh S, Ninov N. Analysis of beta-cell function using single-cell resolution calcium imaging in zebrafish islets. *J Vis Exp*. 2018;**2018**:1–6.
83 Zhao J, Zong W, Zhao Y, Gou D, Liang S, Shen J, et al. In vivo imaging of β-cell function reveals glucose-mediated heterogeneity of β-cell functional development. *Elife*. 2019;**8**:e41540.
84 Helassa N, Podor B, Fine A, Török K. Design and mechanistic insight into ultrafast calcium indicators for monitoring intracellular calcium dynamics. *Sci Rep*. 2016;**6**:38276.
85 Ohkura M, Sasaki T, Kobayashi C, Ikegaya Y, Nakai J. An improved genetically encoded red fluorescent Ca2+ indicator for detecting optically evoked action potentials. *PLoS One*. 2012;**7**:e39933.
86 Lodh S, Hostelley TL, Leitch CC, O'Hare EA, Zaghloul NA. Differential effects on β-cell mass by disruption of Bardet–Biedl syndrome or Alstrom syndrome genes. *Hum Mol Genet*. 2016;**25**:57–68.
87 Cao Z-H, Wu Z, Hu C, Zhang M, Wang W-Z, Hu X-B. Endoplasmic reticulum stress and destruction of pancreatic β cells in type 1 diabetes. *Chin Med J (Engl)*. 2020;**133**:68–73.
88 Engin F. ER stress and development of type 1 diabetes. *J Invest Med*. 2016;**64**:2–6.
89 Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. 2011;**334**:1081–6.
90 Lipson KL, Fonseca SG, Ishigaki S, Nguyen LX, Foss E, Bortell R, et al. Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. *Cell Metab*. 2006;**4**:245–54.
91 Akerboom J, Carreras Calderón N, Tian L, Wabnig S, Prigge M, Tolö J, et al. Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. *Front Mol Neurosci*. 2013;**6**:2.
92 Li J, Chen Z, Gao L-Y, Colorni A, Ucko M, Fang S, et al. A transgenic zebrafish model for monitoring xbp1 splicing and endoplasmic reticulum stress in vivo. *Mech Dev*. 2015;**137**:33–44.
93 Clark EM, Nonarath HJT, Bostrom JR, Link BA. Establishment and validation of an endoplasmic reticulum stress reporter to monitor zebrafish ATF6 activity in development and disease. *Dis Model Mech*. 2019;**13**:dmm041426.
94 Kracht MJL, van Lummel M, Nikolic T, Joosten AM, Laban S, van der Slik AR, et al. Autoimmunity against a defective ribosomal insulin gene product in type 1 diabetes. *Nat Med*. 2017;**23**:501–7.
95 Lenghel A, Gheorghita AM, Vacaru AM, Vacaru A-M. What is the sweetest UPR flavor for the β-cell? That is the question. *Front Endocrinol (Lausanne)*. 2021;**11**:614123.
96 Kanther M, Sun X, Mühlbauer M, Mackey LC, Flynn EJ, Bagnat M, et al. Microbial colonization induces dynamic temporal and spatial patterns of NF-κB activation in the zebrafish digestive tract. *Gastroenterology*. 2011;**141**:197–207.
97 Ellett F, Pase L, Hayman JW, Andrianopoulos A, Lieschke GJ. mpeg1 promoter transgenes direct

macrophage-lineage expression in zebrafish. *Blood*. 2011;**117**:e49–56.
98 Brun T, Maechler P. Beta-cell mitochondrial carriers and the diabetogenic stress response. *Biochim Biophys Acta Mol Cell Res*. 2016;**1863**:2540–9.
99 Breus O, Dickmeis T. Genetically encoded thiol redox-sensors in the zebrafish model: lessons for embryonic development and regeneration. *Biol Chem*. 2021;**402**:363–78.
100 Tao R, Zhao Y, Chu H, Wang A, Zhu J, Chen X, et al. Genetically encoded fluorescent sensors reveal dynamic regulation of NADPH metabolism. *Nat Methods*. 2017;**14**:720–8.
101 Zou Y, Wang A, Shi M, Chen X, Liu R, Li T, et al. Analysis of redox landscapes and dynamics in living cells and in vivo using genetically encoded fluorescent sensors. *Nat Protoc*. 2018;**13**:2362–86.
102 To T-L, Schepis A, Ruiz-González R, Zhang Q, Yu D, Dong Z, et al. Rational design of a GFP-based fluorogenic caspase reporter for imaging apoptosis in vivo. *Cell Chem Biol*. 2016;**23**:875–82.
103 Ham TJ, Mapes J, Kokel D, Peterson RT. Live imaging of apoptotic cells in zebrafish. *FASEB J*. 2010;**24**:4336–42.
104 Zhang Q, Schepis A, Huang H, Yang J, Ma W, Torra J, et al. Designing a Green fluorogenic protease reporter by flipping a Beta Strand of GFP for imaging apoptosis in animals. *J Am Chem Soc*. 2019;**141**:4526–30.
105 Luo X, Li R, Yan L-J. Roles of pyruvate, NADH, and mitochondrial complex I in redox balance and imbalance in β cell function and dysfunction. *J Diabetes Res*. 2015;**2015**:1–12.
106 Keller JP, Marvin JS, Lacin H, Lemon WC, Shea J, Kim S, et al. In vivo glucose imaging in multiple model organisms with an engineered single-wavelength sensor. *Cell Rep*. 2021;**35**:109284.
107 Zhao Y, Hu Q, Cheng F, Su N, Wang A, Zou Y, et al. SoNar, a highly responsive NAD+/NADH sensor, allows high-throughput metabolic screening of anti-tumor agents. *Cell Metab*. 2015;**21**:777–89.
108 Zou Y, Wang A, Huang L, Zhu X, Hu Q, Zhang Y, et al. Illuminating NAD+ metabolism in live cells and in vivo using a genetically encoded fluorescent sensor. *Dev Cell*. 2020;**53**:240–252.e7.
109 Mandal A, Wong H-TC, Pinter K, Mosqueda N, Beirl A, Lomash RM, et al. Retrograde mitochondrial transport is essential for organelle distribution and health in zebrafish neurons. *J Neurosci*. 2021;**41**:1371–92.
110 Tarifeño-Saldivia E, Lavergne A, Bernard A, Padamata K, Bergemann D, Voz ML, et al. Transcriptome analysis of pancreatic cells across distant species highlights novel important regulator genes. *BMC Biol*. 2017;**15**:21.
111 Dybala MP, Gebien LR, Reyna ME, Yu Y, Hara M. Implications of integrated pancreatic microcirculation: crosstalk between endocrine and exocrine compartments. *Diabetes*. 2020;**69**:2566–74.
112 Kimmel RA, Onder L, Wilfinger A, Ellertsdottir E, Meyer D. Requirement for Pdx1 in specification of latent endocrine progenitors in zebrafish. *BMC Biol*. 2011;**9**:75.
113 Choe CP, Choi S-Y, Kee Y, Kim MJ, Kim S-H, Lee Y, et al. Transgenic fluorescent zebrafish lines that have revolutionized biomedical research. *Lab Anim Res*. 2021;**37**:26.
114 Dickmeis T, Feng Y, Mione MC, Ninov N, Santoro M, Spaink HP, et al. Nano-sampling and reporter tools to study metabolic regulation in zebrafish. *Front Cell Dev Biol*. 2019;**7**:15.
115 Panieri E, Millia C, Santoro MM. Real-time quantification of subcellular H 2 O 2 and glutathione redox potential in living cardiovascular tissues. *Free Radic Biol Med*. 2017;**109**:189–200.
116 Bilan DS, Pase L, Joosen L, Gorokhovatsky AY, Ermakova YG, Gadella TWJ, et al. HyPer-3: a genetically encoded H 2 O 2 probe with improved performance for Ratiometric and fluorescence lifetime imaging. *ACS Chem Biol*. 2013;**8**:535–42.
117 Pauls S, Zecchin E, Tiso N, Bortolussi M, Argenton F. Function and regulation of zebrafish nkx2.2a during development of pancreatic islet and ducts. *Dev Biol*. 2007;**304**:875–90.
118 Dalgin G, Prince VE. Mnx1: a gatekeeper of β cell fate. *Islets*. 2012;**4**:320–2.
119 Arkhipova V, Wendik B, Devos N, Ek O, Peers B, Meyer D. Characterization and regulation of the hb9/mnx1 beta-cell progenitor specific enhancer in zebrafish. *Dev Biol*. 2012;**365**:290–302.
120 Ghaye AP, Bergemann D, Tarifeño-Saldivia E, Flasse LC, Von Berg V, Peers B, et al. Progenitor potential of nkx6.1-expressing cells throughout zebrafish life and during beta cell regeneration. *BMC Biol*. 2015;**13**:70.
121 Obholzer N, Wolfson S, Trapani JG, Mo W, Nechiporuk A, Busch-Nentwich E, et al. Vesicular glutamate transporter 3 is required for synaptic transmission in zebrafish hair cells. *J Neurosci*. 2008;**28**:2110–8.
122 Delporte FM, Pasque V, Devos N, Manfroid I, Voz ML, Motte P, et al. Expression of zebrafish pax6b in pancreas is regulated by two enhancers containing highly conserved cis-elements bound by PDX1, PBX and PREP factors. *BMC Dev Biol*. 2008;**8**:53.
123 Huang H, Vogel SS, Liu N, Melton DA, Lin S. Analysis of pancreatic development in living transgenic zebrafish embryos. *Mol Cell Endocrinol*. 2001;**177**:117–24.
124 Zecchin E, Filippi A, Biemar F, Tiso N, Pauls S, Ellertsdottir E, et al. Distinct delta and jagged genes control sequential segregation of pancreatic cell types

from precursor pools in zebrafish. *Dev Biol*. 2007;**301**:192–204.
125 Shin CH, Chung WS, Hong SK, Ober EA, Verkade H, Field HA, et al. Multiple roles for Med12 in vertebrate endoderm development. *Dev Biol*. 2008;**317**:467–79.
126 Wan H, Korzh S, Li Z, Mudumana SP, Korzh V, Jiang Y-J, et al. Analyses of pancreas development by generation of gfp transgenic zebrafish using an exocrine pancreas-specific elastaseA gene promoter. *Exp Cell Res*. 2006;**312**:1526–39.
127 Park SW, Davison JM, Rhee J, Hruban RH, Maitra A, Leach SD. Oncogenic KRAS induces progenitor cell expansion and malignant transformation in zebrafish exocrine pancreas. *Gastroenterology*. 2008;**134**:2080–90.