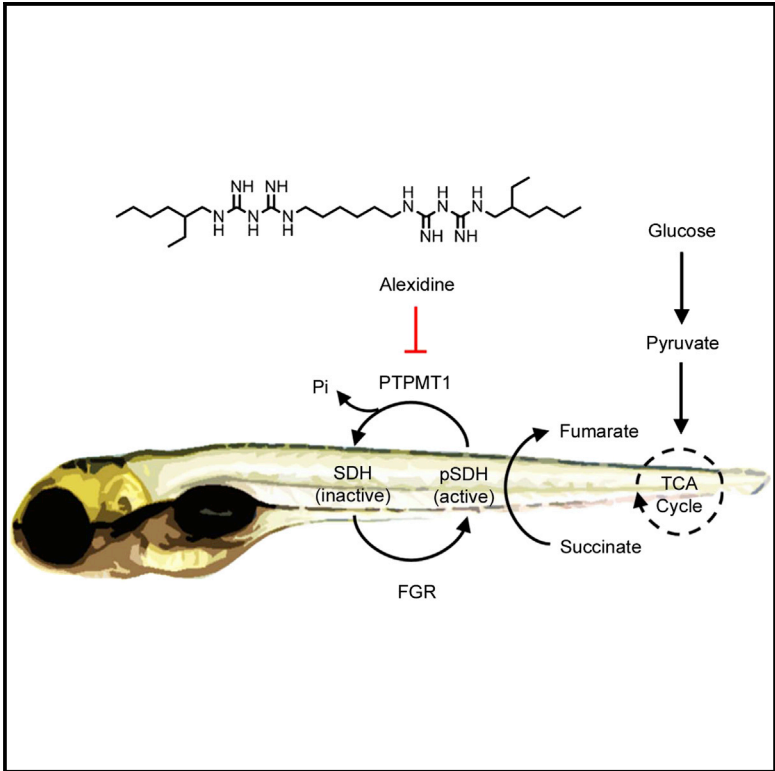


## PTPMT1 Inhibition Lowers Glucose through Succinate Dehydrogenase Phosphorylation

### Graphical Abstract



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### In Brief

Nath et al. use chemical screening in larval zebrafish to uncover pathways that lower glucose. The authors find that inhibition of the mitochondrial-specific phosphatase PTPMT1 leads to increased phosphorylation and activation of succinate dehydrogenase. These findings provide a possible mechanism by which PTPMT1 coordinates glucose utilization by the mitochondria.

### Highlights

- An in vivo chemical screen identifies alexidine as a glucose-lowering agent
- Alexidine targets PTPMT1 to lower glucose
- Phosphorylated succinate dehydrogenase (SDH) is a PTPMT1 substrate
- Alexidine increases SDH activity in vivo



# PTPMT1 Inhibition Lowers Glucose through Succinate Dehydrogenase Phosphorylation

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## SUMMARY

Virtually all organisms seek to maximize fitness by matching fuel availability with energy expenditure. In vertebrates, glucose homeostasis is central to this process, with glucose levels finely tuned to match changing energy requirements. To discover new pathways regulating glucose levels *in vivo*, we performed a large-scale chemical screen in live zebrafish and identified the small molecule alexidine as a potent glucose-lowering agent. We found that alexidine inhibits the PTEN-like mitochondrial phosphatase PTPMT1 and that other pharmacological and genetic means of inactivating PTPMT1 also decrease glucose levels in zebrafish. Mutation of *ptpmt1* eliminates the effect of alexidine, further confirming it as the glucose-lowering target of alexidine. We then identified succinate dehydrogenase (SDH) as a substrate of PTPMT1. Inactivation of PTPMT1 causes hyperphosphorylation and activation of SDH, providing a possible mechanism by which PTPMT1 coordinates glucose homeostasis. Therefore, PTPMT1 appears to be an important regulator of SDH phosphorylation status and glucose concentration.

## INTRODUCTION

Glucose homeostasis is closely regulated in animals as diverse as flies and humans (Haselton and Fridell, 2010). Although much is known about the central role of endocrine hormones in glucose homeostasis, we have only a superficial understanding of the processes that coordinate fuel availability and utilization (Stanley et al., 2014). Conversion of carbon substrates into energy occurs predominately in the mitochondria. Recent explorations of mitochondrial biology have revealed that this ancient organelle does much more than simply convert fuel substrates into energy (Cui et al., 2010; McBride et al., 2006; Tait and Green, 2012). The discovery of an extensive catalog of phosphoproteins, kinases, and phosphatases in the mitochondrial proteome

has revolutionized our concept of the mitochondria (Cui et al., 2010; Goldenthal and Marín-García, 2004; Pagliarini et al., 2008; Zhao et al., 2011). The observation that mitochondria possess the components to sense and process signals from the environment by reversible phosphorylation implies that protein phosphorylation networks within the mitochondrion play an important role in orchestrating the bioenergetics of the cell. However, questions remain regarding which phosphorylated metabolic enzymes coordinate energy availability and utilization.

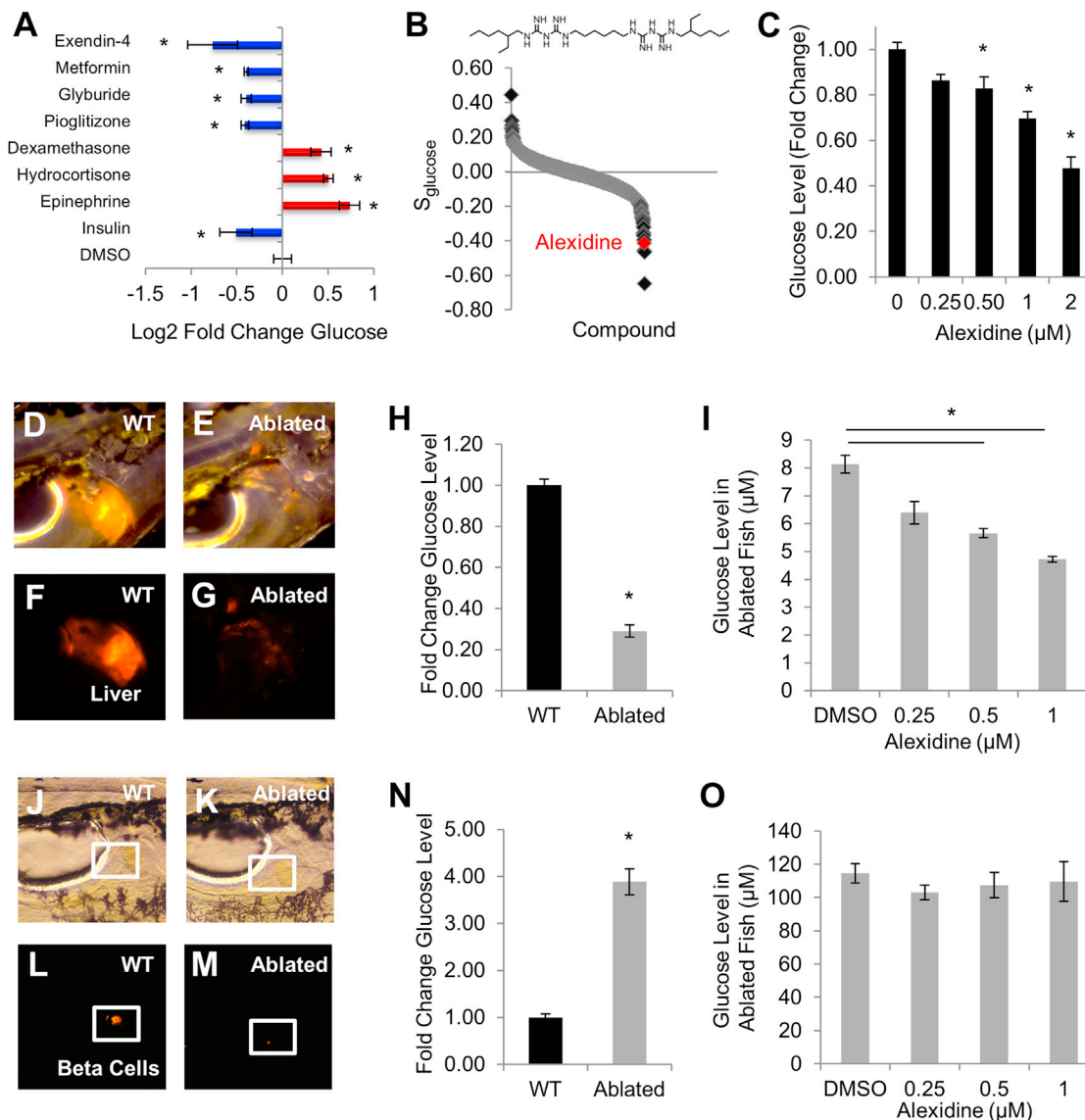
One likely focal point for mitochondrial signaling is succinate dehydrogenase (SDH) (also known as respiratory complex II). SDH is an integral membrane protein complex within the mitochondrion that catalyzes the oxidation of succinate to fumarate and delivers the resulting electrons to coenzyme Q<sub>10</sub>. SDH is unique compared to other mitochondrial respiratory complexes in that it participates in both the electron transport chain and the tricarboxylic acid cycle, placing it in an ideal position to affect mitochondrial metabolism. The biochemical transformations SDH mediates are well known, but its regulation remains poorly understood (Rutter et al., 2010). Emerging evidence identifies FGR as a kinase that phosphorylates the catalytic subunit of SDH (SDHA), but the cognate phosphatase and any other signaling regulators remain unknown (Acín-Pérez et al., 2014; Salvi et al., 2007).

Here, we use a large-scale chemical screen in zebrafish to identify the small molecule alexidine as a potent glucose-lowering agent. We show that alexidine targets the mitochondrial-specific phosphatase PTPMT1 and that both chemical and genetic perturbations of PTPMT1 lower glucose levels in a whole organism. We identify tyrosine-phosphorylated SDHA as a substrate of PTPMT1 and demonstrate that PTPMT1 inhibition modulates SDHA phosphorylation status. Collectively, our results point to PTPMT1 phosphatase activity as a regulatory mechanism for SDH enzymatic activity and as an important node for glucose homeostasis.

## RESULTS AND DISCUSSION

### Known Gluconeogenic Hormones and Antidiabetic Drugs Modulate Glucose Homeostasis in Zebrafish

To determine if zebrafish larvae could be used for discovering novel pathways regulating glucose homeostasis, we evaluated



**Figure 1. In Vivo Chemical Screen for Modifiers of Glucose Levels Identifies Alexidine as a Glucose-Lowering Agent**

(A) Measurement of glucose levels in larval zebrafish after treatment with known hyper- and hypoglycemic agents ( $n = 3$ ).

(B) S-score (log ratio of the fold change in glucose levels) ranking of the glucose-lowering ability of compounds in the Prestwick library. Alexidine's score is depicted in red, and its chemical structure is shown on the graph.

(C) Alexidine dose-response curve ( $n = 5$ ).

(D–G) Bright-field (D and E) and fluorescent (F and G) images of wild-type livers (D and F) and ablated livers (E and G).

(H) Glucose measurements in larvae with wild-type livers versus ablated livers.

(I) Glucose measurements in alexidine-treated larvae with ablated livers ( $n = 3$ ).

(J–M) Bright-field (J and K) and fluorescent (L and M) images of wild-type (J and L)  $\beta$  cells and ablated  $\beta$  cells (K and M).

(N) Glucose measurements in larvae with wild-type  $\beta$  cells versus ablated  $\beta$  cells.

(O) Glucose measurements in alexidine-treated larvae with ablated  $\beta$  cells ( $n = 3$ ).

Data are presented as mean  $\pm$  SEM. \* $p < 0.01$ . See also Figure S1.

their response to known human hyperglycemic and hypoglycemic agents. Day 5 zebrafish larvae were treated with various agents for 4–8 hr and harvested for glucose measurements. Insulin, extendin-4, or the antidiabetic drugs pioglitazone, glyburide, or metformin significantly decreased glucose levels in zebrafish ( $-50\% \pm 17\%$ ,  $p = 0.03$ ;  $-76\% \pm 27\%$ ,  $p = 0.05$ ;

$-41\% \pm 4\%$ ,  $p < 0.001$ ;  $-39\% \pm 5\%$ ,  $p = 0.002$ ;  $-39\% \pm 2\%$ ,  $p < 0.001$  respectively; Figure 1A), as they do in humans. Conversely, treatment with epinephrine, hydrocortisone, or dexamethasone significantly increased glucose levels in zebrafish ( $73\% \pm 10\%$ ,  $p < 0.001$ ;  $50\% \pm 5\%$ ,  $p < 0.001$ ;  $42\% \pm 11\%$ ,  $p = 0.04$ , respectively), similar to their effects in humans.

### Whole-Organism Chemical Screen for Modifiers of Glucose Levels Identifies Alexidine as a Potent Glucose-Lowering Agent

Given that hormones and drugs that are known to change glucose levels in humans also increase or decrease glucose levels in zebrafish in a predictable manner, we sought to use the zebrafish as a tool for identifying novel chemical modulators of glucose homeostasis. We developed a high-throughput assay that measures systemic glucose levels in larval zebrafish by coupling glucose oxidase to a horseradish peroxidase reaction. We screened 13,120 compounds for their effects on glucose levels in 78,720 larval zebrafish. Among these, one of the most potent glucose-lowering compounds was alexidine (Figure 1B), a bisguanide compound with antimicrobial properties that was not previously known to decrease glucose levels. In confirmation studies, alexidine was found to significantly reduce glucose levels in a dose dependent manner (Figure 1C). To rule out a generalized developmental or toxicity effect, 0 hr postfertilization embryos and 5 days postfertilization (5 dpf) larvae were treated with alexidine for 24 hr. No morphological defects or changes in viability were observed (Figures S1A–S1F). Additionally, larvae were treated with alexidine for 8 hr followed by the addition of TMRM, a dye that detects mitochondrial membrane potential and is one indicator of intact mitochondrial function. Compared to treatment with a chemical uncoupler of electron transport and oxidative phosphorylation, DNP-2, alexidine did not cause a notable change in mitochondrial membrane potential, indicating functional mitochondria (Figures S1G–S1I).

### The In Vivo Glucose-Lowering Effect of Alexidine Treatment Is Dependent on $\beta$ Cells

Organismal glucose levels are regulated by multiple organs, but two common sites of drug action are the liver (the primary site of gluconeogenesis) and pancreatic  $\beta$  cells (the primary site of insulin production). To discriminate between the possibilities that alexidine was acting on the liver or pancreas, we leveraged liver and pancreas ablation models generated in zebrafish. Hepatocytes were ablated using a transgenic line (*lfabp*:GAL4-VP16; UAS.nfsB-mCherry) in which nitroreductase (NTR) is expressed by the liver fatty acid binding protein promoter (Figures 1D and 1F). Upon addition of metronidazole, liver cells expressing NTR convert the prodrug into a cytotoxic compound that leads to cell death. At 24 hr posttreatment, the majority of hepatocytes in the transgenic zebrafish larvae were destroyed (Figures 1E and 1G), resulting in a significant  $\sim$ 3-fold decrease in glucose levels (Figure 1H;  $-71\% \pm 3\%$ ;  $p < 0.001$ ). Nevertheless, after ablation of hepatocytes, alexidine retained the ability to induce a decrease in glucose levels, with an effect size indistinguishable from that in unablated animals (Figure 1I). These data suggest that the liver is not required for alexidine's hypoglycemic effect. By contrast,  $\beta$  cells were ablated using a transgenic line in which NTR is driven by the insulin promoter (*ins*:nfsB-mCherry) (Figures 1J and 1L). At 24 hr postablation, near-complete loss of  $\beta$  cells was observed (Figures 1K and 1M), which resulted in a significant  $\sim$ 4-fold increase in glucose levels (Figure 1N;  $3.88 \pm 0.07$ ;  $p < 0.001$ ). In the absence of  $\beta$  cells, alexidine was unable to decrease glucose levels (Figure 1O), suggesting that  $\beta$  cells are required for alexidine's glucose-lowering effect.

### Structurally Distinct PTPMT1 Inhibitors Phenocopy Alexidine's Glucose-Lowering Effect

Next, we sought to determine the molecular mechanism by which alexidine modulates glucose levels. A prior study had shown that alexidine can inhibit PTPMT1, the only known protein tyrosine phosphatase that resides exclusively in the mitochondria (Doughty-Shenton et al., 2010). PTPMT1 is one of the most highly conserved phosphatases known with orthologs in four phylogenetic kingdoms. However, to date, little is known about the substrates and the biological functions of PTPMT1 (Pagliarini et al., 2005; Shen et al., 2011; Zhang et al., 2011). To determine if PTPMT1 inhibition is responsible for the reduction in glucose levels induced by alexidine, we first confirmed alexidine's ability to inhibit PTPMT1 in vitro and found that alexidine inhibits PTPMT1 with a half maximal inhibitory concentration of 1  $\mu$ M (Figure 2A). Subsequent in vivo studies with chlorhexidine (CLX), a PTPMT1 inhibitor structurally similar to alexidine, resulted in decreased glucose levels ( $-28\% \pm 4\%$ ;  $p < 0.004$ ; Figures 2B and 2C). Next, we evaluated the effects of a panel of structurally distinct PTPMT1 inhibitors on glucose levels in vivo (Figure 2B) (Park et al., 2012). Four structurally distinct PTPMT1 inhibitors (compounds 3–6) lowered glucose levels in zebrafish to a degree that was comparable to alexidine ( $-28\% \pm 5\%$ ,  $-32\% \pm 5\%$ ,  $-31\% \pm 4\%$ ,  $-25\% \pm 4\%$ ;  $p < 0.04$ ; Figure 2D). The finding that several structurally distinct PTPMT1 inhibitors all lower glucose levels supports the hypothesis that PTPMT1 is the glucose-lowering target of alexidine.

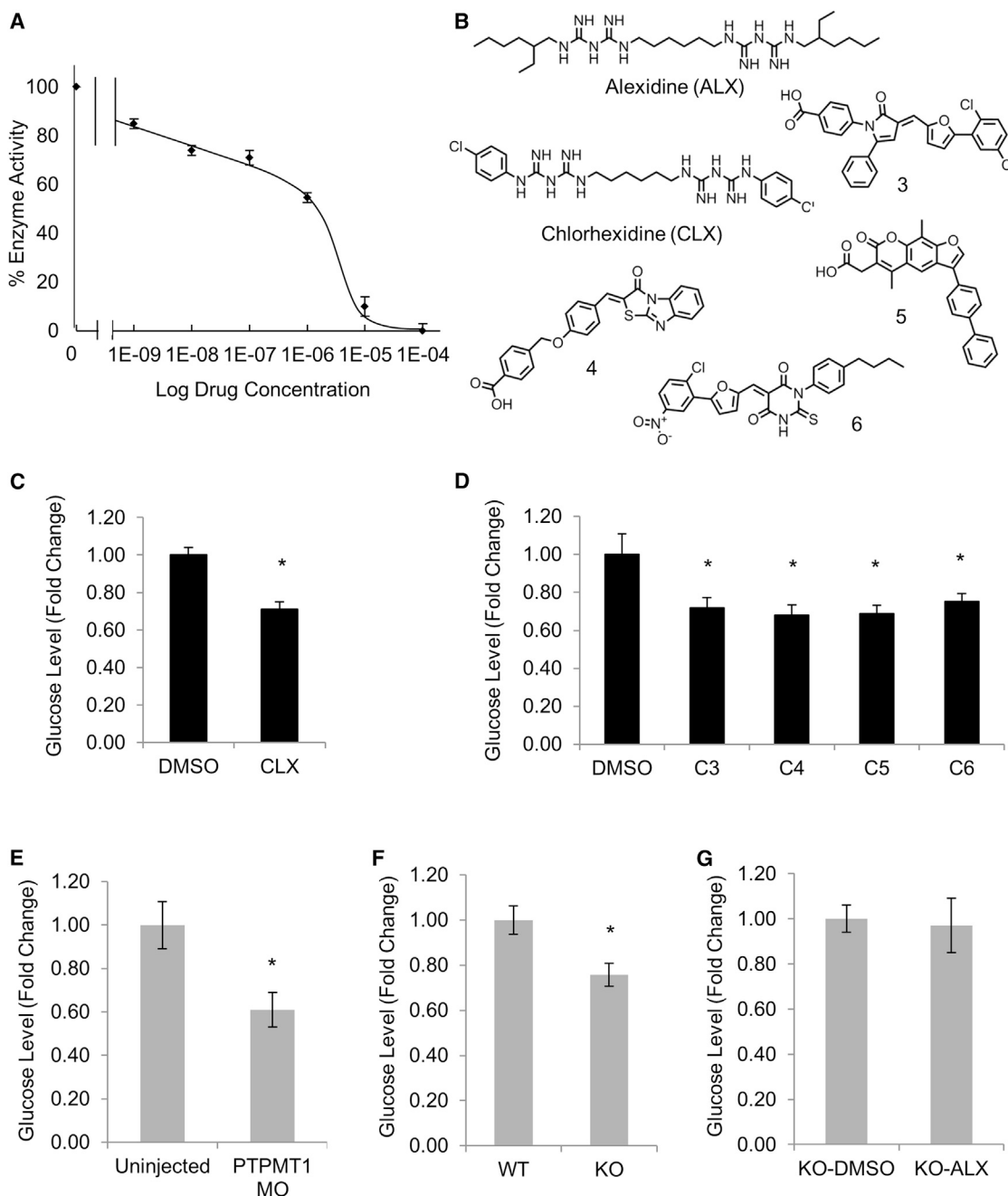
### PTPMT1 KO Larvae Are Hypoglycemic

To complement our chemical studies, we evaluated the effect of knocking down *ptpmt1* gene function by using a translation blocking antisense morpholino oligonucleotide (MO). At  $\sim$ 50% knockdown, *ptpmt1* morphants were morphologically normal but exhibited significantly decreased glucose levels ( $-39\% \pm 7\%$ ;  $p = 0.02$ ) compared to uninjected controls (Figures S2A–S2C and 2E). We also sought to determine the effect of true genetic *ptpmt1* knockout (KO). Using TALEN-mediated gene targeting, we generated a *ptpmt1* KO line (Figures S2D–S2M). KOs develop mitochondrial defects at  $\sim$ 14 dpf and die at  $\sim$ 30 dpf (Figures S2I–S2M). No KOs survived to adulthood. However, 6 dpf larvae KO display normal gross morphology and normal mitochondria (Figures S2D–S2H). *ptpmt1*<sup>-/-</sup> larvae displayed significantly decreased glucose levels compared to wild-type controls (Figure 2F), indicating that *ptpmt1* loss of function is sufficient to lower glucose levels in vivo.

Given that small molecules often have multiple targets, we also used the *ptpmt1*<sup>-/-</sup> larvae to verify the specificity of our findings. We tested whether alexidine retained its glucose-lowering activity in the absence of *ptpmt1* by treating *ptpmt1*<sup>-/-</sup> animals with alexidine. Alexidine failed to lower glucose levels in *ptpmt1*<sup>-/-</sup> animals (Figure 2G). Together, these data suggest that PTPMT1 is the relevant target of alexidine and is necessary for alexidine's glucose-lowering effect.

### Succinate Dehydrogenase Is a Substrate for PTPMT1

PTPMT1's apparent ability to alter glucose levels made us curious as to the identity of its substrates. Previously, phospholipid profiling of *ptpmt1*<sup>-/-</sup> fibroblasts led to the discovery that



**Figure 2. PTPMT1 Is the Glucose-Lowering Target of Alexidine**

(A) Alexidine dose-response effect on PTPMT1 phosphatase activity.

(B) Panel of PTPMT1 inhibitors that are structurally similar (chlorhexidine) or structurally distinct (3–6) from alexidine.

(C and D) Glucose measurements in larvae treated with (C) chlorhexidine or (D) compounds 3–6.

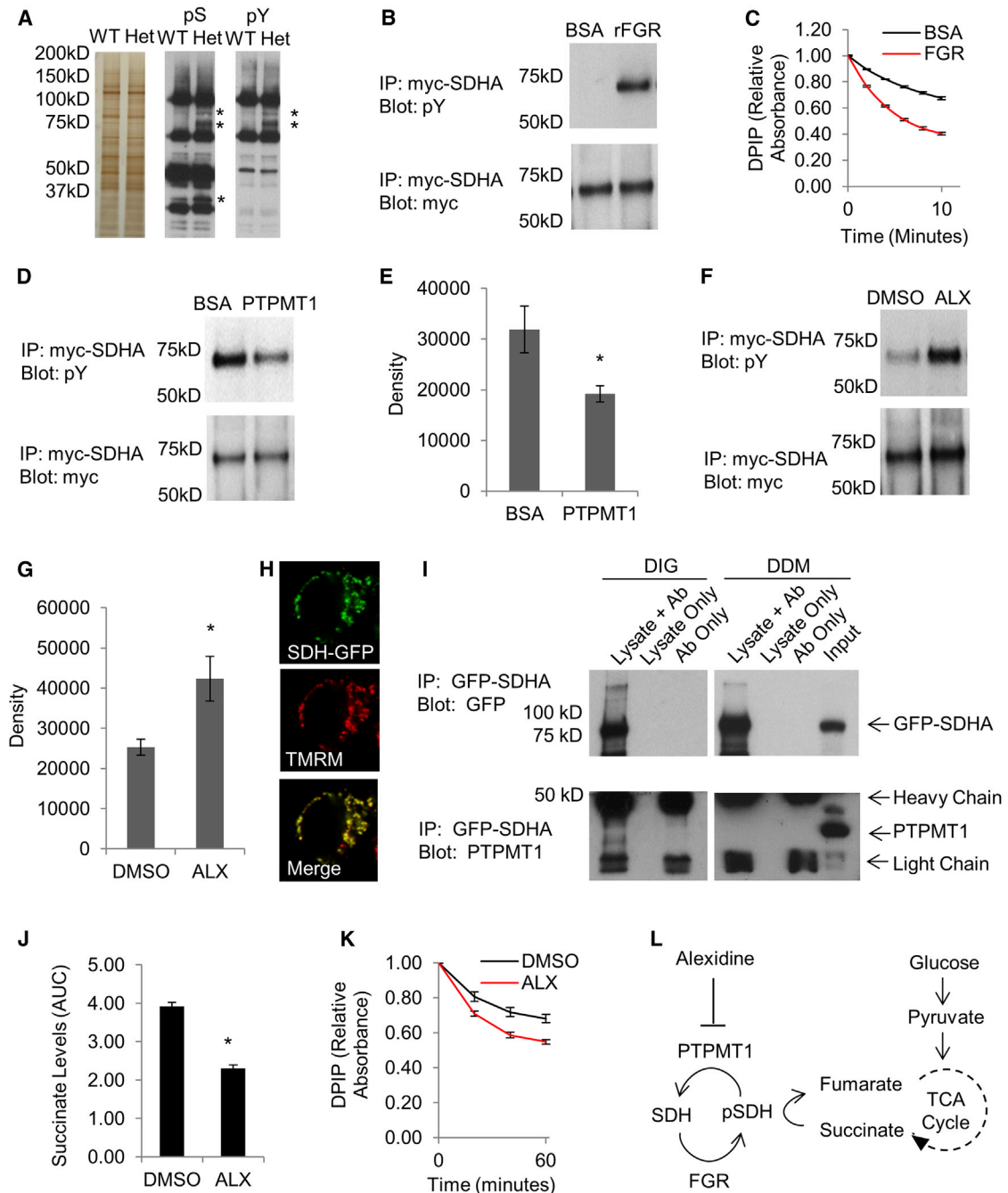
(E and F) Glucose measurements in (E) larvae injected with *ptpmt1* MO or (F) wild-type (WT) versus *ptpmt1*<sup>-/-</sup> larvae (KO).

(G) Glucose measurements in *ptpmt1*<sup>-/-</sup> larvae treated with DMSO or alexidine.

Data are presented as mean ± SEM. \*p < 0.01. See also Figure S2.

PTPMT1 dephosphorylates phosphatidylglycerophosphate, an essential intermediate step in the biosynthesis of cardiolipin (Zhang et al., 2011). At the same time, PTPMT1 belongs to the dual-specificity phosphatase family, suggesting that its

glucose-regulating mechanism might also involve protein substrates. In an effort to determine if PTPMT1 has phosphoprotein substrates in vivo, we compared phosphoprotein profiles in zebrafish lysates from wild-type and *ptpmt1* mutant animals.



**Figure 3. SDH Is a Substrate for PTPMT1**

(A) Phosphotyrosine and phosphoserine western blots on lysates from wild-type or *ptpmt*<sup>+/-</sup> livers.  
 (B) Western blots from myc-SDHA-transfected INS-1E cell lysates subjected to incubation with FGR, followed by immunoprecipitation for myc and western blot for phosphotyrosine.  
 (C) SDH enzymatic activity assay on mitochondrial lysates treated with BSA or rFGR (SD = 0.005; p < 0.0001).  
 (D) Western blots for phospho-tyrosine and myc from lysates treated with rPTPMT1.  
 (E) Quantification of n = 3 western blots.  
 (F) Western blots for phosphotyrosine and myc from lysates treated with alexidine.  
 (G) Quantification of n = 3 western blots.  
 (H) Mitochondrial localization of SDHA in a HEK GFP-SDHA stable line.  
 (I) Western blots of SDHA-PTPMT1 coimmunoprecipitation experiments in cells lysed with a mild detergent (DIG) versus a stronger detergent (DDM). Controls included antibody only and lysate only. The last lane contained total cell lysate.

(legend continued on next page)

Although homozygous *ptpmt1* mutants do not survive to adulthood, heterozygous animals do survive and were used for phosphoprotein comparisons. Lysates from *ptpmt1*<sup>+/-</sup> adults displayed several proteins with increased phosphorylation relative to wild-types, including a prominent ~80 kDa protein that was dramatically hyperphosphorylated in *ptpmt1*<sup>+/-</sup> animals (Figure 3A). The induction of phosphoprotein changes in *ptpmt1* mutants motivated us to take a candidate-based approach to identifying potential PTPMT1 protein substrates.

Given PTPMT1's exclusive location within the inner mitochondrial membrane, we hypothesized that PTPMT1 might regulate a mitochondrial metabolic pathway. This hypothesis was of particular interest because succinate dehydrogenase (SDH) resides on the inner mitochondrial membrane where PTPMT1 localizes. SDH participates in both the electron transport chain and the tricarboxylic acid cycle, placing it in an ideal position to affect mitochondrial metabolism. Furthermore, the SDH subunit SDHA is approximately the same size as the protein that is hyperphosphorylated in *ptpmt1* mutants. A conserved phosphorylation site was recently discovered on SDHA that can be phosphorylated by FGR kinase and is physiologically relevant for mitochondrial function (Acín-Pérez et al., 2014; Salvi et al., 2007). Given these observations, we hypothesized that SDHA is a substrate of PTPMT1 and that phosphorylation of SDHA activates the enzyme leading to increased succinate utilization.

To determine if SDHA is a substrate for PTPMT1, lysates from myc-SDHA-transfected INS-1E  $\beta$  cells were treated with BSA or the tyrosine kinase FGR in vitro followed by immunoprecipitation of myc-SDHA and western blotting for phosphotyrosine. Phosphorylation of SDHA at baseline was undetectable; however, compared to BSA, FGR potently phosphorylated SDHA (Figure 3B). Next, we sought to determine if FGR-mediated phosphorylation has functional effects on SDH activity. To do so, we incubated phosphorylated SDH with purified FGR or an equivalent amount of BSA and then assessed SDH activity levels using a colorimetric activity assay. FGR-incubated mitochondrial lysates exhibited increased enzymatic activity relative to controls, suggesting that phosphorylation of SDH increases SDH activity (Figure 3C). This finding is consistent with a previous report showing that FGR can phosphorylate Y604 on SDHA (Acín-Pérez et al., 2014).

To determine if phosphorylated SDHA is a substrate of PTPMT1, lysates from myc-SDHA-transfected INS-1E cells were treated with the tyrosine kinase FGR in the presence of rPTPMT1 or BSA. Compared to control-treated lysates, PTPMT1 significantly decreased tyrosine phosphorylation of SDHA in FGR-treated lysates (Figures 3D and 3E). Next, mitochondrial lysates were treated with FGR in the presence of DMSO or alexidine to determine if inhibition of PTPMT1 affects SDHA phosphorylation. Alexidine treatment resulted in a ~2-fold increase in the level of pY-SDHA compared to mitochondrial extracts treated with DMSO (Figures 3F and 3G).

To determine if PTPMT1 interacts with SDHA, we performed coimmunoprecipitation experiments in a human embryonic kidney (HEK) cell line that stably expresses GFP-SDHA (Figure 3H). PTPMT1 coimmunoprecipitated with SDHA, suggesting a physical interaction exists between these two proteins (Figure 3I). The interaction was disrupted by using a stronger detergent, indicating a transient or weak interaction between the two proteins as is commonly observed in phosphatase-substrate interactions (Figure 3I). Reversing the target used for pull-down recapitulated that PTPMT1 and SDHA interact (Figures S3A–S3F). Finally, mutating tyrosine residue 604 of SDHA decreased the interaction between PTPMT1 and SDHA (Figures S3C–S3F). Collectively, these data demonstrate that pY-SDHA is a substrate of PTPMT1 in vitro.

### Chemical Inhibition of PTPMT1 Decreases Succinate Levels via Increased SDH Activity In Vivo

Having observed that SDH is a substrate for PTPMT1 and that phosphorylation of SDH increases its activity, we sought to determine if PTPMT1 inhibition could cause a significant change in succinate levels in vivo. We performed a targeted liquid chromatography-tandem mass spectrometry scan that analyzed 48 intermediary metabolites in zebrafish treated with alexidine. We found that alexidine treatment caused a highly significant decrease in succinate levels ( $p = 4.3 \times 10^{-5}$ ; Figure 3J). Therefore, the PTPMT1 inhibitor alexidine causes a decrease in succinate levels in vivo that correlates with the increased SDH activity it causes in vitro. To confirm that the decrease in succinate is due to increased consumption of succinate rather than a block earlier in the tricarboxylic acid cycle, SDH activity was measured in mitochondria isolated from zebrafish larvae treated with alexidine or DMSO. Alexidine treatment increased the rate of succinate dehydrogenase activity by 59% ( $0.00125 \pm 0.00007$  to  $0.00212 \pm 0.00003$   $\Delta$ OD/min;  $p = 0.0003$ ), demonstrating that inhibition of PTPMT1 increases the rate of SDH activity in vivo (Figure 3K).

Collectively, these studies suggest a model in which (1) alexidine inhibits the phosphatase activity of PTPMT1, (2) FGR activity leads to the accumulation of the phosphorylated form of SDHA, (3) enzymatic activity of SDH increases, and (4) glucose levels decrease (Figure 3L). The finding that alexidine ultimately activates SDH provides an explanation for why it is so effective at lowering glucose levels. Increased SDH activity may lower glucose levels in at least two complementary ways: by stimulating the uptake of glucose and by increasing the rate of glucose utilization.

Interestingly, previous work has demonstrated that *ptpmt1* knockdown in INS-1E  $\beta$  cells results in increased insulin secretion in vitro (Pagliarini et al., 2005). By linking PTPMT1 and SDH, our data provide a possible mechanism by which PTPMT1 inhibition causes insulin secretion. Consumption of succinate by SDH is known to induce insulin secretion, a

(J) Succinate levels in zebrafish larvae treated with DMSO or alexidine.

(K) SDH activity in mitochondria from zebrafish larvae treated with alexidine (SD = 0.02,  $p = 0.0003$ ).

(L) Collectively, these studies suggest a model in which (1) alexidine inhibits the phosphatase activity of PTPMT1, (2) FGR activity leads to accumulation of the phosphorylated form of SDHA, (3) enzymatic activity of SDH increases, and (4) glucose levels decrease.

Data are presented as mean  $\pm$  SD. \* $p < 0.01$ . See also Figure S3.

phenomenon described by “the succinate theory” (Alarcon et al., 2002). Therefore, inhibition of PTPMT1 may lower glucose levels by engaging succinate metabolism to promote insulin secretion. Currently, the methodology to collect blood from a 5 dpf larva and measure blood insulin concentrations does not exist, making it difficult to determine directly if alexidine induces insulin secretion in zebrafish. Nevertheless, our data demonstrate that after  $\beta$  cell ablation in zebrafish, alexidine was unable to decrease glucose levels, providing additional support for the idea that insulin secretion plays some role in alexidine’s mechanism of action.

Our ablation data support an insulin-mediated pathway for alexidine’s effects. One caveat, however, is that peripheral tissues such as muscle require insulin for insulin-mediated glucose uptake. Therefore, the ablation model may also be consistent with a mechanism of action involving glucose utilization. Interestingly, inhibition of PTPMT1 increases the ATP/ADP ratio (Pagliarini et al., 2005). Further, skeletal muscle expresses high levels of PTPMT1. Therefore, a second complementary mechanism by which a PTPMT1-SDH connection may regulate glucose levels is altering the rate of glucose utilization. Phosphorylation of SDH has recently been identified as a regulatory point in cellular adaptation to changing nutrient demands (Acín-Pérez et al., 2014). Reactive oxygen species-driven activation of SDH by FGR affects SDH activity, supercomplex distribution in the mitochondria, and metabolic adaptation of mitochondria to starvation or hypoxia/reoxygenation (Acín-Pérez et al., 2014). Therefore, in addition to increasing glucose uptake by stimulating insulin secretion, PTPMT1 inhibition may increase the rate of glucose utilization by stimulating SDH activity. By establishing a link between PTPMT1 and SDH, we not only describe a new function for a poorly understood phosphatase (PTPMT1) but also provide a molecular regulatory mechanism for SDH, an enzyme fundamental to mitochondrial function and the coordinated uptake and utilization of glucose. Furthermore the identification of PTPMT1 as a target for augmenting SDH provides new possibilities for modulating glucose homeostasis. A better understanding of this pathway might ultimately lead to new opportunities to intervene pharmacologically in diabetes and other metabolic disorders.

## EXPERIMENTAL PROCEDURES

### Zebrafish

Animals were maintained and embryos were obtained according to standard fish husbandry protocols in accordance with the Massachusetts General Hospital Institutional Animal Care and Use Committee. See [Supplemental Experimental Procedures](#) for details.

### Chemical Screen

The Prestwick (1,120), Spectrum (2,000), and ChemBridge (10,000) libraries were screened at a concentration of  $\sim 8 \mu\text{g/ml}$ . The assay was carried out on larval zebrafish loaded into 96-well plates. Viability was assessed by observing heart rate, and glucose was measured using the Amplex Red Glucose Assay Kit (Invitrogen) according to the manufacturer’s instructions. Product formation was determined by reading fluorescence emission at 595 nm. All other compounds were purchased from Sigma-Aldrich. PTPMT1 inhibitors were synthesized from Sigma-Aldrich (Park et al., 2012).

### Phosphatase Assay

PTPMT1 activity was measured using the EnzChek Phosphatase Assay Kit (Invitrogen). The assay buffer contained 100  $\mu\text{M}$  DiFMUP, 20 mM Na acetate, 5 mM Bis Tris, 5 mM Tris, 5% DMSO, and 22 nM rPTPMT1 (pH 7.0). Measurements were taken every 2 min for 60 min at 358 nm excitation/455nm emission.

### Metabolic Profiling

See [Supplemental Experimental Procedures](#) for details.

### Morpholino Oligonucleotide Injections

A *ptpmt1* translation-blocking MO (GeneTools) was used (5'-TTCTCGCT AAAACACTCGACATGGT-3'). MO was injected into the yolk at the one-cell stage. Knockdown was quantified by western blot.

### Cell Culture

INS-1E cells (a kind gift from Dr. Pierre Maechler, Geneva University) were cultured in RPMI-1640 supplemented with 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.05 mM 2-mercaptoethanol, 5% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100  $\mu\text{g/ml}$  streptomycin (Merglen et al., 2004). HEK cells were grown in DMEM, 10% FBS.

### SDH Activity Assay

Whole-cell lysates were prepared by incubating the cell pellet in 10% n-dodecyl  $\beta$ -D-maltoside for 30 min followed by centrifugation at  $20,000 \times g$  for 10 min. Alternatively, mitochondria were prepared by differential centrifugation in mitochondrial buffer B. SDH activity was measured in cell lysates or whole mitochondria using 2,6-dichlorophenolindophenol according to the Rosen method (Rosen et al., 1987).

### Kinase Assay

See [Supplemental Experimental Procedures](#) for details.

### Statistical Analyses

All results are expressed as means  $\pm$  SD or SEM. A two-tailed Student’s t test was used to determine p values.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.01.010>.

## AUTHOR CONTRIBUTIONS

A.K.N. and R.T.P. contributed to conception and design, acquisition of data, interpretation of data, and drafting of the manuscript. Y.N.J., L.D.R., A.D., and J.H.R. contributed to the acquisition and interpretation of data. R.E.G. contributed to the interpretation of data and critical revision of draft.

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