Supporting Information

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SI Text

Zebrafish Strains and Lines. Zebrafish were raised under standard laboratory conditions at 28 °C. Heat-shocks were performed at 39.5 °C. We used the following lines: *Tg(XlEef1a1:GFP)*^{s854} (1), *Tg(−4.0ins:GFP)*^{zf5} (2), *Tg(ins:dsRed)*^{m1018} (3), *Tg(Ptf1a:eGFP)*^{jh1} (4), *Tg(ins:Cre; cryaa:Venus)*^{s924}; *Tg(hsp70l:loxP-mCherry-STOP-loxP-H2B-GFP; cryaa:Cerulean)*^{s923} (defined here as "Insulin-HOTcre"), *Tg(ins:CFP-NTR)*^{s892} (5), and *prkci*^{m567} (6).

DNA Constructs and Transgenic Lines. *ins-Cre*; *cryaa:Venus* was generated by placing the *Cre* coding sequence downstream of 1.5 kb of the proximal insulin promoter. The *cryaa:Venus* cassette was inserted downstream of *Cre* in the reverse orientation using 0.54 kb of the *cryaa* promoter (7). *hsp70l:loxP-mCherry-STOP-loxP-H2B-GFP*; *cryaa:Cerulean* was constructed using 1.5 kb of the *hsp70l* promoter (8). The floxed *mCherry-STOP* and *H2B-GFP* fragments were PCR amplified individually and sequentially cloned downstream of the *hsp70l* promoter. The mCherry-STOP cassette serves as a heat-shock induction marker prevents read-through translation of the H2B-GFP fusion protein, which is only expressed in cells that have undergone a Cre mediated excision event. *cryaa:Cerulean* was inserted downstream of *H2B-GFP* in the reverse orientation. Both constructs were generated in a pBluescript backbone that contains I-*Sce*I meganuclease sites. Multiple transgenic lines were established for each construct using the I-*Sce*I meganuclease method as described in ref. 9. A single representative transgenic line for each construct was used for all experiments.

Immunofluorescence. Antibody staining was performed as described in ref. 10 using the following antibodies: Islet-1 (1:100, Developmental Studies Hybridoma Bank clone 39.4D5), insulin (1:100, Biomeda V2024), somatostatin (1:100, Serotec 8330–0154), glucagon (1:200, Sigma G2654), GFP (1:500, Aves Labs GFP-1020 or 1:500, Stratagene 632380), and Alexa secondary antibodies (1:500, Invitrogen). Cell nuclei were visualized with the DRAQ5 DNA stain (1:1000, Biostatus Limited DR50050) or TOPRO3 (1:2000, Invitrogen T3605).

Morpholino Injections. We injected 8 ng of a previously described morpholino targeted against the translational start site of *prkci* (11).

Proliferation Analysis. For EdU incorporation and detection, we used the Click-IT 647 kit (Invitrogen C10085) according to manufacturer's instructions, with adaptations for whole mount samples. Briefly, tricaine-anesthetized larvae were injected into the yolk or transpericardially with approximately 5 nL EdU solution (1 μM/ 2% DMSO/0.1% phenol red), then recovered at 28 °C. Samples were fixed overnight in 3% formaldehyde in PBS and de-yolked. Samples were then treated with PBS + 0.3% Triton X-100 for 20 min, rinsed once with ddH20, and then reacted with 250 μL fresh labeling solution for 20 min.

FACS Analysis. Two hundred larvae at 12 dpf were enriched for islets by removing the head and tail with a scalpel. For FACS analysis at 24 hpf the yolk, head, and tail were removed from 200 embryos with fine gauge needles. The samples were dissociated in 1 mL 5% FBS/HBSS with Liberase 3 (Roche) at 37 °C for 1 h. The cells were filtered three times with a 40-μM nylon strainer and sorted using the FACSDiva platform (BD Biosciences).

Real-Time RT-PCR. RNA was isolated from 0.5–2 $\times$ 10^4 cells using TRIzol (Invitrogen 15596). cDNA was prepared using the SuperScript III kit (Invitrogen). Optimized primers targeting each gene and β-actin were designed using the Plexor Primer Design System (Promega, Table S2). cDNA was amplified with Power SYBR Green master mix (Applied Biosystems). The 7900HT Real-Time PCR System (Applied Biosystems) was used to obtain Ct values. The relative expression of each sample was determined after normalization to β-actin using the relative standard curve method (12).

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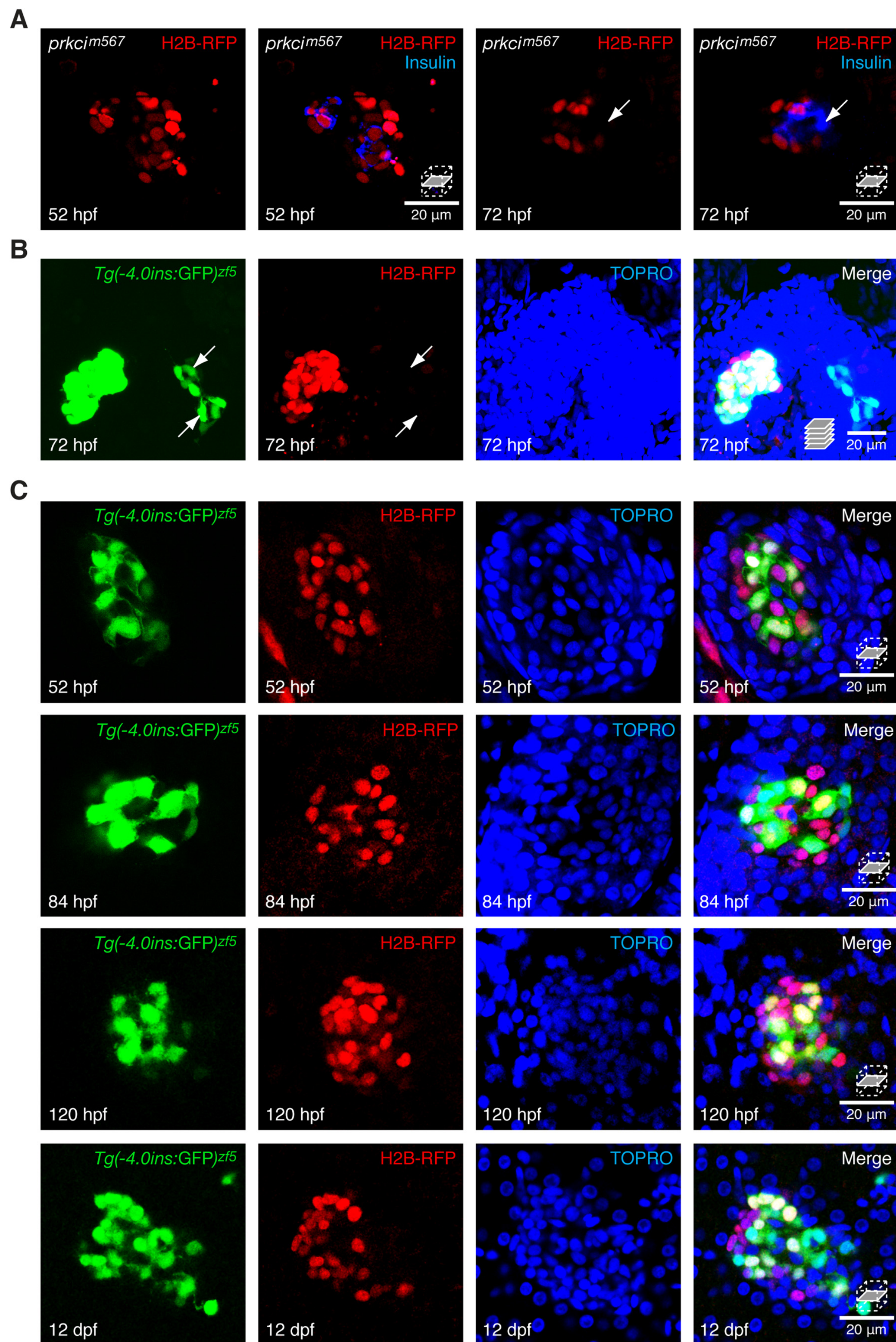

Fig. S1. H2B-RFP is retained in dorsal bud derived β-cells during embryonic and larval development. All embryos were injected with H2B-RFP mRNA at the one cell stage. (*A*) Confocal sections of *prkci*^{m567} mutants, at 52 and 72 hpf, stained with insulin. (*B*) Confocal projection of an islet stained with TOPRO. (*C*) Confocal sections of islets that express *Tg*(−*4.0ins*:GFP)^{zf5} at different developmental time-points stained with TOPRO. (*A*) All insulin-expressing cells retain the H2B-RFP label at 52 hpf in *prkci*^{m567} mutants. By 72 hpf, label diluted VBCs appear (arrow). (*B*) *Tg*(−*4.0ins*:GFP)^{zf5}-positive cells (arrows) that differentiate outside the principal islet do not exhibit the H2B-RFP label. (*C*) H2B-RFP label-retaining endodermal cells can be clearly distinguished from the surrounding label-diluted cells by 52 hpf. The H2B-RFP label remains easily detectable at 12 dpf.

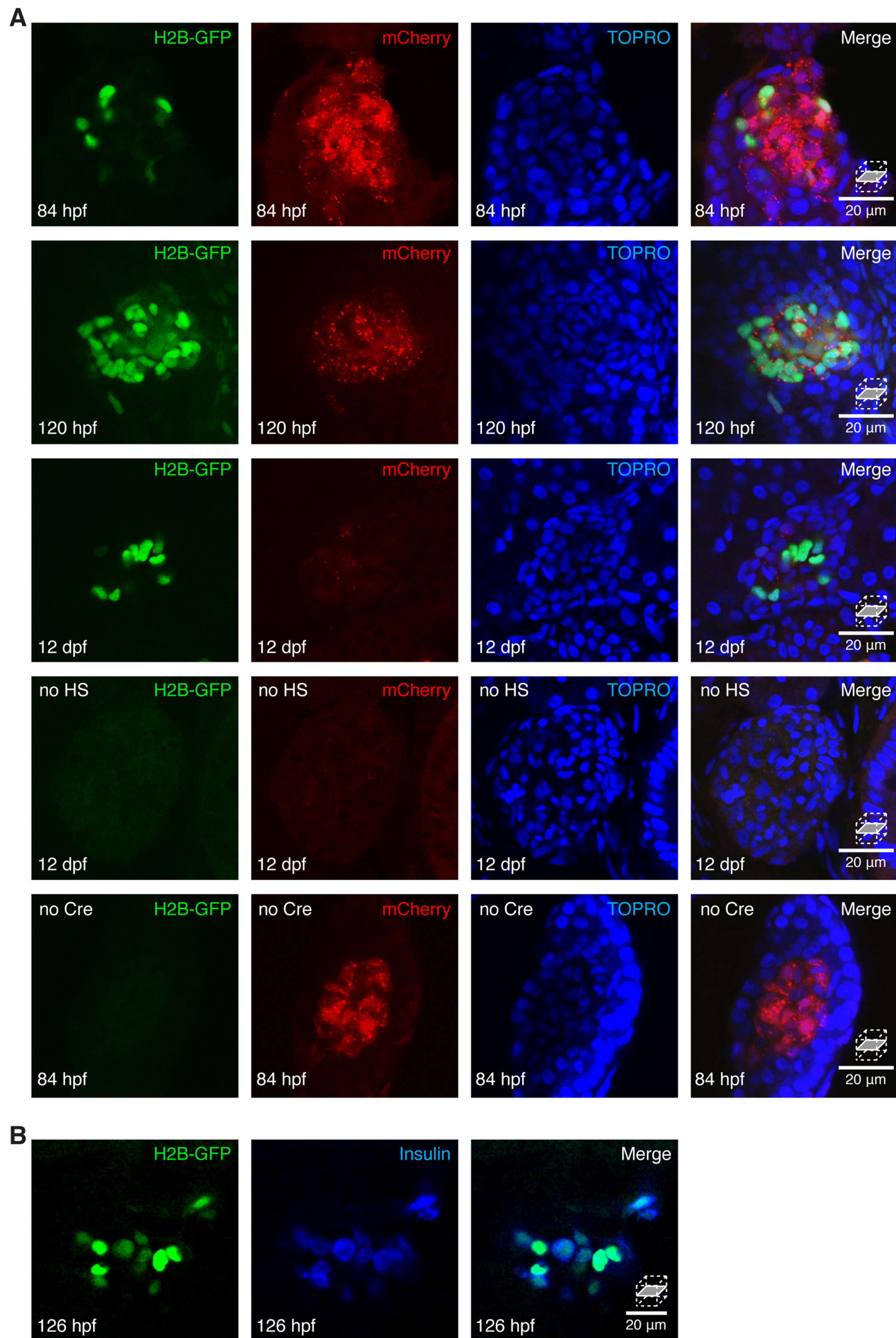

Fig. S2. HOTcre controls. (*A* and *B*) Confocal sections of islets stained with TOPRO (*A*) to mark nuclei. (*A*) The mCherry induction reporter was induced ubiquitously and H2B-GFP was expressed specifically in β-cells by heat-shock of Insulin-HOTcre embryos at 24 hpf. By 84 hpf, mCherry is expressed at low levels in many tissues. Within the slowly dividing endocrine compartment, mCherry is initially retained as bright puncta. The intensity of the mCherry signal is reduced by 120 hpf and reaches the limit of detection by 12 dpf. Without heat-shock treatment, H2B-GFP is not detected in the islet. In the absence of *Tg*(*ins*:*Cre*; *cryaa*:*Venus*)^{s924}, H2B-GFP is not detected in the islet following heat-shock treatment at 24 hpf. (*B*) H2B-GFP was expressed specifically in β-cells by heat-shock of Insulin-HOTcre embryos at 120 hpf. By 126 hpf, H2B-GFP is expressed in all Insulin positive cells.

Table S1. Islet quantification

Sample			n
Figure 2F	GFP(+) dsRed(-)	GFP(+) dsRed(+)	
24 hpf	21.0 ± 1.3	0	10
28 hpf	27.1 ± 1.6	0	8
34 hpf	26.3 ± 1.7	0	8
40 hpf	31.1 ± 0.9	20.1 ± 1.6	7
46 hpf	33.6 ± 1.3	25.8 ± 1.0	10
Figure 2H	GFP(+) H2B-RFP(+)	Total GFP(+)	
52 hpf	22.0 ± 0.6	31.8 ± 1.0	12
84 hpf	21.7 ± 1.9	34.5 ± 1.0	11
120 hpf	21.0 ± 1.0	35.6 ± 1.2	9
12 dpf	22.4 ± 1.7	61.6 ± 3.3	10
Figure 3D	H2B-GFP(+)		
30 hpf	21.0 ± 1.1		9
84 hpf	19.5 ± 1.3		6
120 hpf	21.7 ± 1.6		6
12 dpf	22.7 ± 1.2		7
Figure 4B	H2B-GFP(+) H2B-RFP(+)	H2B-GFP(+)H2B-RFP(-)	
126 hpf	21.5 ± 1.2	11.0 ± 3.2	10
12 dpf	22.2 ± 1.8	19.4 ± 1.9	10
Figure 4D	GFP(+)CFP(+)	GFP(-)CFP(+)	
126 hpf	32.8 ± 1.1	0	10
12 dpf	41.9 ± 1.3	20.1 ± 0.9	10

Table S2. Real-time RT-PCR primers

Gene	Accession	Primers
β-actin	NM_131031	GTGGTCTCGTGGATACCGCAA
		CTATGAGCTGCCTGACGGTCA
insulin	NM_131056	TTTAAATGCAAAGTCAGCCACCTCAG
		GGCTTCTTCTACAACCCCAAGAGAGA
pdx1	NM_131443	GGGCGCGAGATGTATTTGTTGA
		CAAATCTCACACGCACGCATG
neuroD	NM_130978	TCATGCTTTCCTCGCTGTATGACT
		CCACGAAGGGCATGAAACTATCA
mafl	NM_001082940	GGCAGAAACTTGAGACTCGTGAA
		CTCTAAGGACGATTTCTCTGCAACC
nkx2.2a	NM_131422	TCAGCTGAAGGTTCCGGAGATTTT
		CGACAATCCTTATACTAGATGGCTCG
mafba	NM_131015	AACTGCAAAATGCCCGTTATTGG
		GCAGACCTACAAACCGACATCG
mafbb	NM_131842	CTTCACATCAAACCTCATGTCAAAGT
		GCAGAAATCATGTCGGCGGAG
cdkn1a	XM_001923789	CGCTTCTTGGCTTGGTAGAAATCT
		ATCCCGAAAACACCAGAACGAT
cdkn1b	NM_212792	GAAGCGACGACAATGACGACAT
		CGCCCAGAAAACCCAAAAAACC
cdkn1bl	XM_686521	TTGTATTTTGGAAGCGCCGTCT
		GCTCCCTGCGAACCGGACC
cdkn1c	NM_001008595	CATCCGCTCTGCAGATAAACACA
		GCACATCCACGAGCATTGAAGT
cdkn3	XM_001331893	CGCTCTTTGGTTTCCTGATATGCTT
		CCGGAGCCATTCAGACTGTCA
ptf1a	NM_207641	CAGTCCATGAAAGAGAGTGTCCT
		CCTTAAACCAACCGAAAAAAGTCATCATT

Table S3. Raw Ct values for real-time RT-PCR experiments

Gene	Ct value (DBCs at 12 dpf) ± SEM	Ct value (VBCs at 12 dpf) ± SEM
β-actin	24.1 ± 0.1	24.1 ± 0.1
insulin	31.1 ± 0.1	24.7 ± 0.1
pdx1	34.4 ± 0.4	31.5 ± 0.1
neuroD	37.6 ± 0.4	36.2 ± 0.2
mafl	33.3 ± 0.2	32.9 ± 0.2
pax6b	36.1 ± 0.2	33.5 ± 0.2
nkx2.2a	35.2 ± 0.1	34.1 ± 0.3
mafba	31.9 ± 0.1	34.5 ± 0.2
mafbb	31.0 ± 0.1	33.5 ± 0.4
cdkn1a	31.0 ± 0.1	31.7 ± 0.2
cdkn1b	30.8 ± 0.1	31.5 ± 0.2
cdkn1bl	33.4 ± 0.4	34.3 ± 0.3
cdkn1c	33.8 ± 0.1	35.7 ± 0.5
cdkn3	DNA	DNA
ptf1a	DNA	DNA

DNA = Did not amplify.