SUPPLEMENTARY DATA

Inhibition of Cdk5 Promotes β-cell Differentiation from Ductal Progenitors

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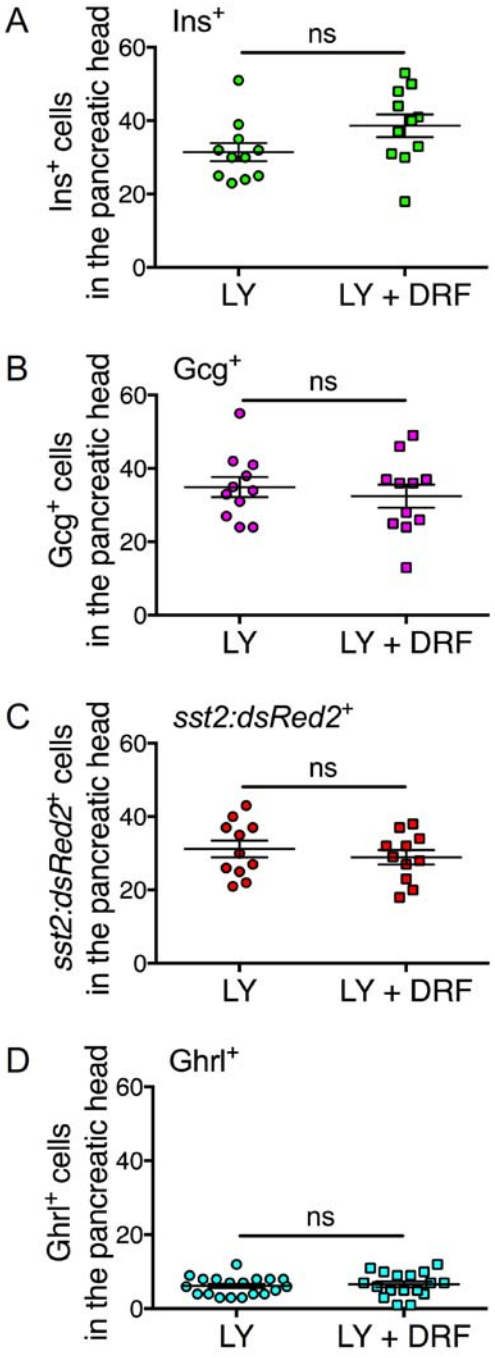
Supplementary Figure S1. Chemical inhibition of Cdk5 does not significantly change the number of endocrine cells in the pancreatic head region. Quantification of insulin-positive (A), glucagon-positive (B), *sst2:dsRed2*$^{+}$ (C) and ghrelin-positive (D) cells in the pancreatic head per larva at 6 dpf after treatment with LY in the presence or absence of DRF from 3 – 6 dpf; n = 11 – 19.



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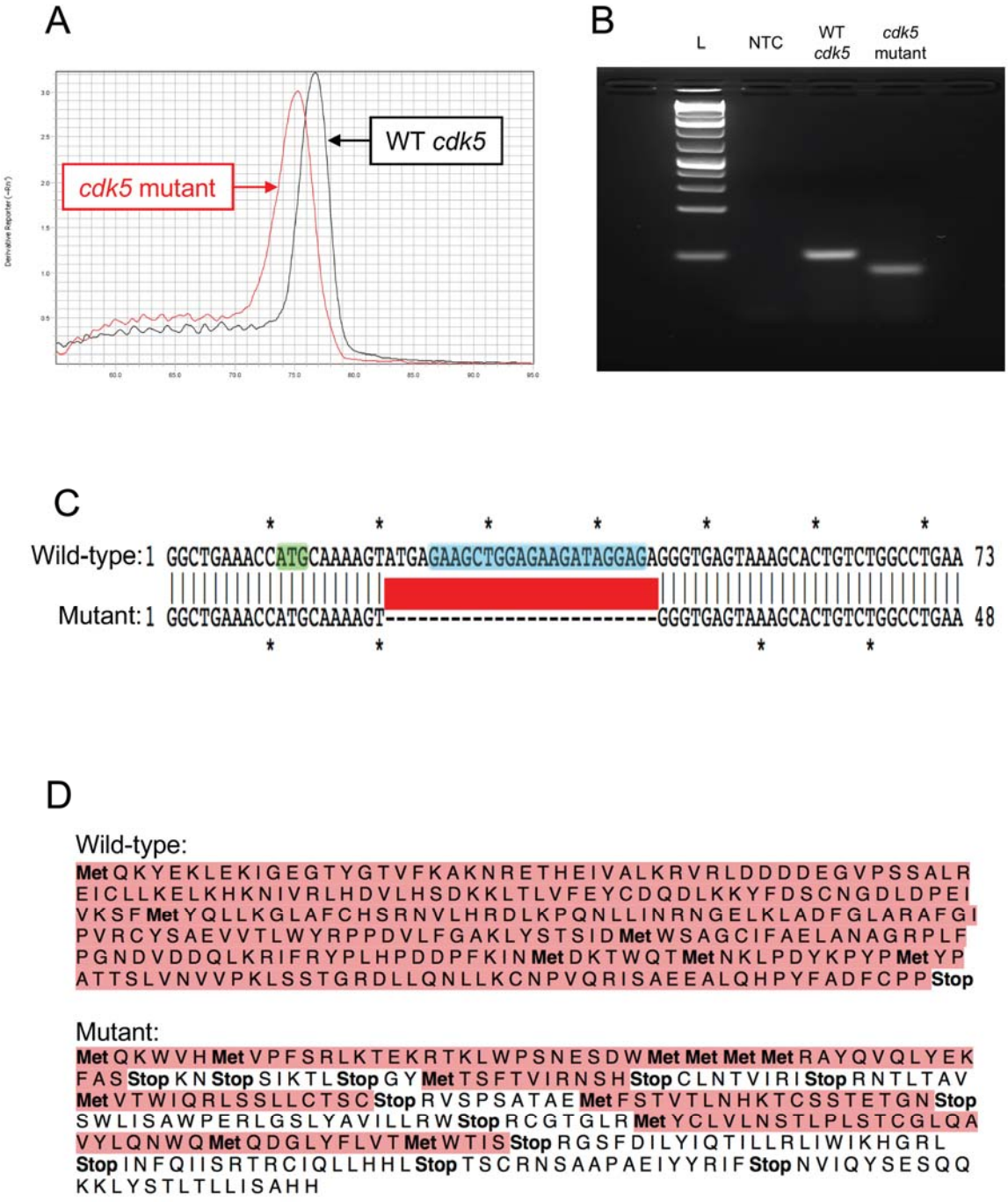
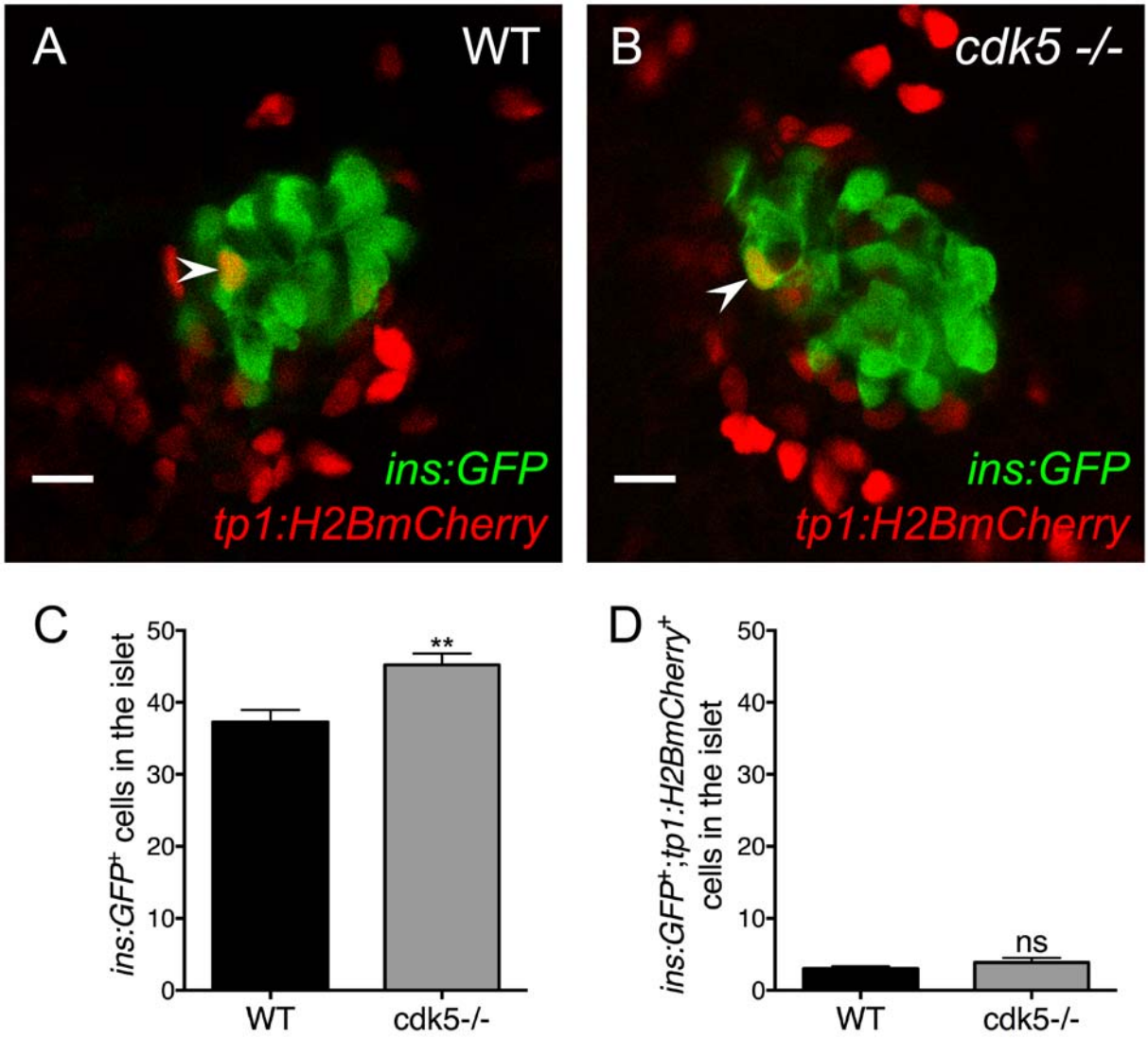
Supplementary Figure S2. The *cdk5* mutant carries a 25-bp deletion that induces a frameshift. (A) Melting curves of real-time PCR products from genomic DNA template of WT and *cdk5* mutant. (B) Gel electrophoresis image showing the size difference between the real-time PCR products of WT and *cdk5* mutant. L: O'GeneRuler 1 kb Plus DNA Ladder; NTC: no-template control. (C) Alignment of *cdk5* nucleotides from WT zebrafish larvae and *cdk5* mutants. Red: region of deletion in mutants; Green: start codon of *cdk5*; Blue: targeting sequence of sgRNA. (D) Prediction of Cdk5 protein sequences after translation from the WT and mutated transcripts processed by Translate Tool (SIB Swiss Institute of Bioinformatics). Open reading frames are highlighted in red.



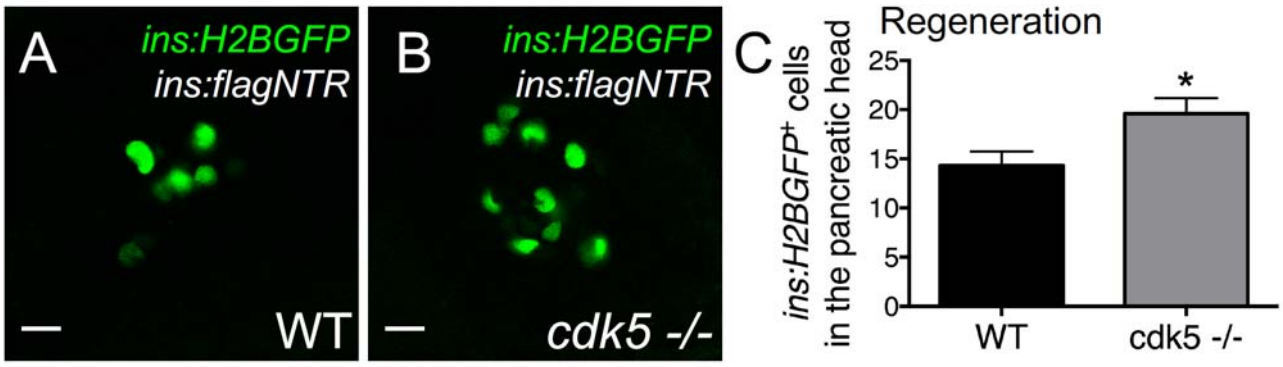
SUPPLEMENTARY DATA

Supplementary Figure S3. The increased number of β-cells in the pancreatic head of *cdk5* mutants does not originate from *tp1*$^+$ ductal cells. (A and B) Representative confocal images of the pancreatic head region of WT and *cdk5*-/- zebrafish larvae carrying *Tg(ins:GFP);Tg(tp1:H2BmCherry)* at 6 dpf, displaying β-cells in green and β-cells with a notch-responsive ductal lineage in yellow overlap (arrowheads). Scale bar = 10 μm. Quantification of *ins:GFP*$^+$ cells (C) and *ins:GFP*$^+$; *tp1:H2BmCherry*$^+$ co-expressing cells (D) in the pancreatic head at 6 dpf. $^{**}P \leq 0.01$; $n = 14 – 17$.



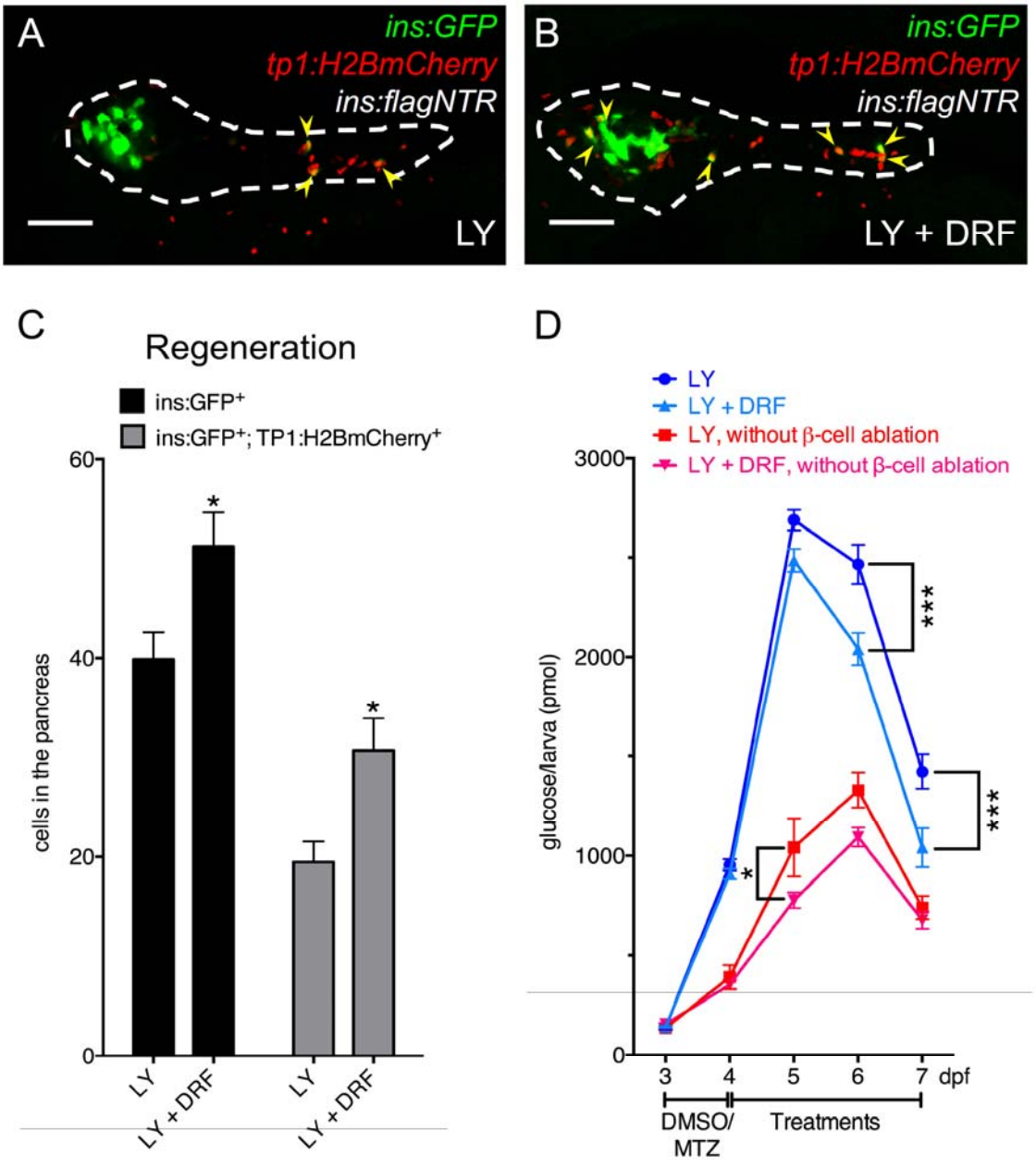
SUPPLEMENTARY DATA

Supplementary Figure S4. Deletion of *cdk5* promotes regeneration of β-cells. (A and B) Representative confocal images of the pancreatic head of WT and *cdk5*-/- zebrafish larvae carrying *Tg(ins:H2BGFP);Tg(ins:flagNTR)* at 6 dpf, displaying β-cells in green. Scale bar = 10 μm. (C) Quantification of *ins:H2BGFP*$^{+}$ cells in the head of the pancreas per larva at 6 dpf after β-cell ablation. **P* ≤ 0.05; n = 9 – 12.



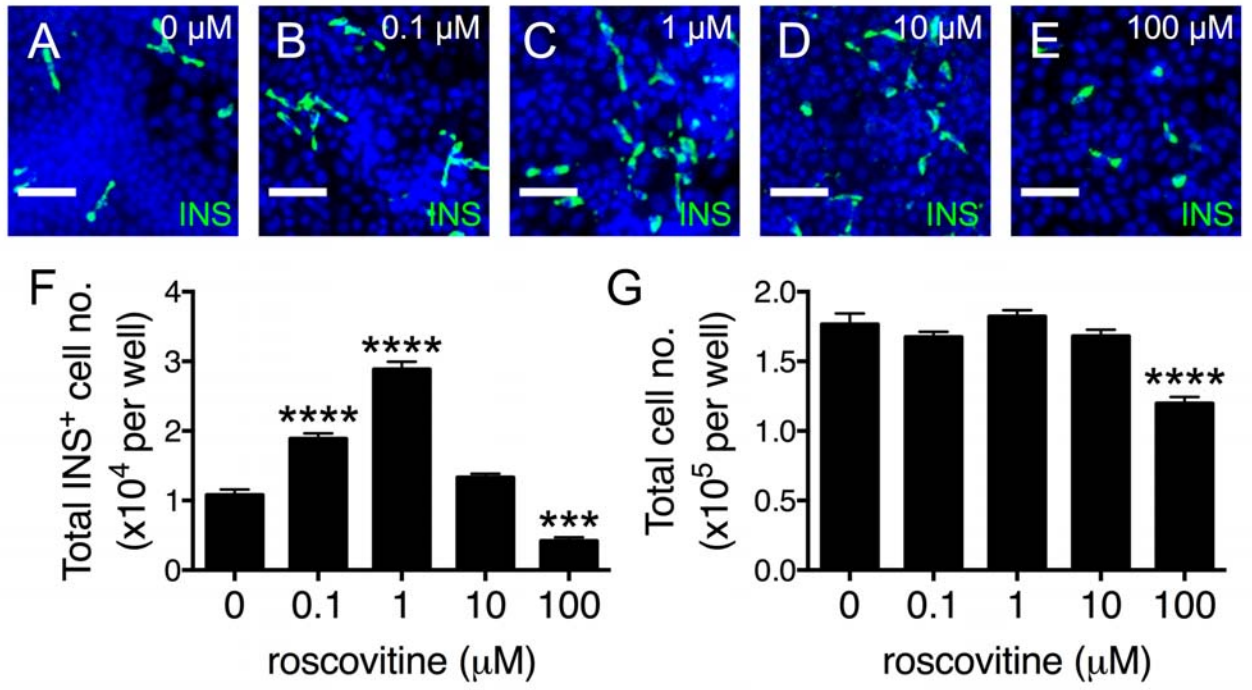
SUPPLEMENTARY DATA

Supplementary Figure S5. Inhibition of Cdk5 facilitates regeneration of β-cells and restoration of glycemia after β-cell depletion. (A – C) DRF promoted β-cell regeneration. (A and B) Representative confocal images of the pancreas of *Tg(ins:GFP);Tg(tp1:H2BmCherry);Tg(ins:flagNTR)* larvae at 6 dpf incubated with MTZ from 3 to 4 dpf to ablate β-cells and treated with LY or LY plus DRF from 4 to 6 dpf, displaying β-cells in green and β-cells originating from the notch-responsive ductal lineage in yellow overlap (arrowheads). Dashed lines show the outline of the pancreas. Scale bar = 50 μm. (C) Quantification of regenerated *ins:GFP*$^{+}$ and *ins:GFP*$^{+}$; *tp1:H2BmCherry*$^{+}$ β-cells per larva at 6 dpf. *$P \leq 0.05$; $n = 15$. (D) Free-glucose level of *Tg(ins:kaede);Tg(ins:CFP-NTR)* zebrafish larvae from 3 to 7 dpf. The larvae were incubated with DMSO or MTZ from 3 to 4 dpf and treated with LY or LY plus DRF from 4 to 7 dpf. Free-glucose levels of larvae without ablating the β-cells are shown for baseline reference. *$P \leq 0.05$ and ***$P \leq 0.001$; $n = 28$ larvae (four groups of seven pooled larvae) per data point.



SUPPLEMENTARY DATA

Supplementary Figure S6. Roscovitine enhances β-cell differentiation from human iPS cells in a dose-dependent manner. (A – E) Representative images of human iPS cells treated with roscovitine at 0, 0.1, 1, 10 and 100 μM during Stage 4, displaying INS^+ cells in green and DAPI in blue. Scale bar = 50 μm. (F) Total number of INS^+ cells per well. ***$P \leq 0.001$ and ****$P \leq 0.0001$; $n = 3$. (G) Total number of cells per well. ****$P \leq 0.0001$; $n = 3$.



SUPPLEMENTARY DATA

Supplementary Figure S7. Treatment with roscovitine at Stage 5 promotes differentiation of human iPS cells to β-cells. (A) Schema for differentiating human iPS cells to insulin-producing cells. The cells were treated with DMSO or roscovitine at Stage 5. (B and C) Representative images of human iPS cells treated with DMSO or roscovitine at Stage 5, displaying INS^+ cells in green and DAPI in blue. Scale bar = 50 μm. (D) Percentages of differentiated human iPS cells expressing insulin after treatment with DMSO or roscovitine at Stage 5. $*P \leq 0.05$; $n = 3$.