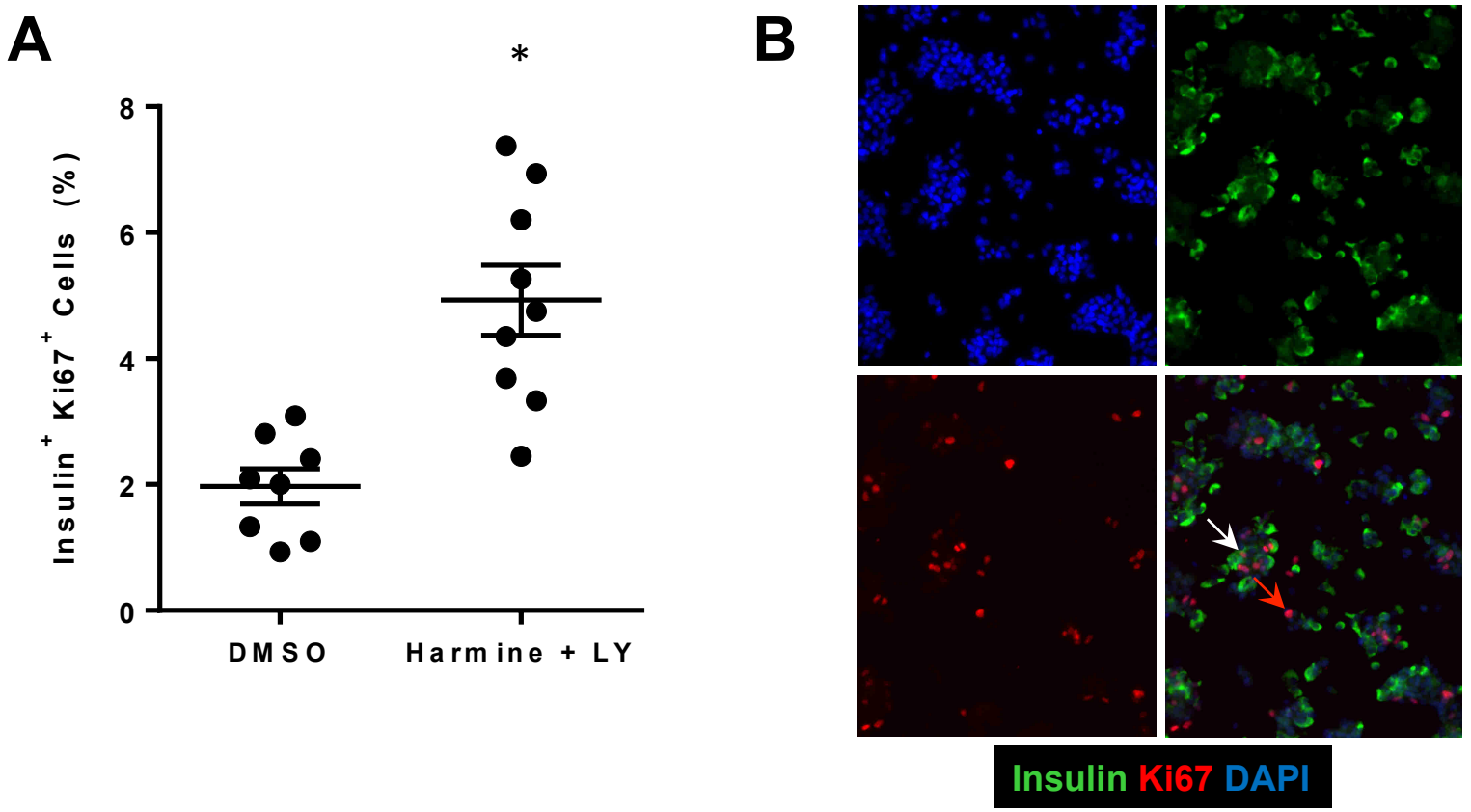

Figure S1. (Related to Fig. 1) Synergistic Effects of Harmine Plus TGFβ Receptor Inhibition on HUES8 Human Pluripotent Stem Cell-Derived Beta Cell Proliferation. **A. H**armine-TGFβ inhibitor synergy in HUES8-derived beta cells treated with the DYRK1A inhibitor harmine (10 μM) plus a TGFβ receptor (TGF-β RI, ALK5) inhibitor LY364947 (LY, 1 μM) for four days. Drug treatment significantly increased beta cell proliferation by 2.5-fold. Each dot represents a replicate from a different HUES8-derived beta cell differentiation batch. Three different HUES8 batches were used. Bars indicate mean ± SEM. * indicates $p<0.007$ **B.** DAPI, Insulin, Ki67 and merged images from an example of overnight Ki67 and insulin immunofluorescence labeling of HUES8-derived beta cells in Stage 6 of culture. Note that beta cells and non-beta cells are induced to proliferate: the white arrow highlights a $Ki67^+$ beta cell. The red arrow marks a $Ki67^+$ non-beta cell.

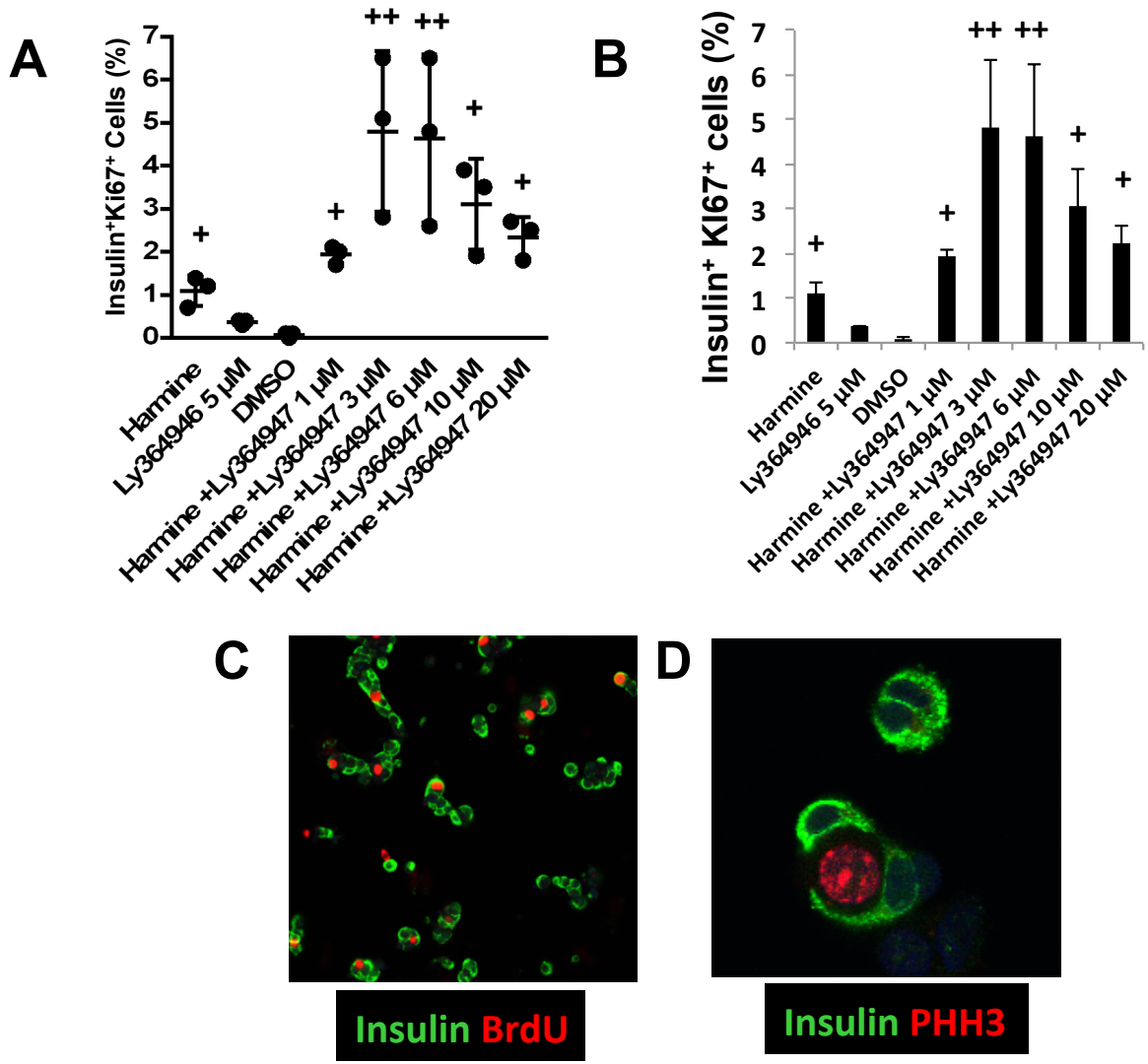

Figure S2. (Related to Fig. 1) Additional Evidence for Synergistic Effects of Harmine-TGFβ Receptor Inhibition on Human Beta Cell Proliferation. A. Formal demonstration of harmine-TGFβ inhibitor synergy. A maximally effective dose of harmine, 10 µM (1) induces low rates of proliferation, while TGFβ inhibition with LY364947 has no effect. In contrast, when modestly effective doses of harmine are combined with progressively larger doses of the LY compound, marked dose-related increases in human beta cell proliferation are observed. Each dot represents a different normal islet donor. $^{+}$indicates $p<0.05$ vs. control, and $^{++}$indicates $p<0.05$ vs. harmine. Bars indicate mean ± SEM. **B.** Identical data to panel A, presented as a bar graph. $^{+}$symbols are as in Panel A, and bars indicate mean ± SEM. **C.** An example of overnight BrdU immunolabeling of dispersed whole islets, to compliment Figure 1F. **D.** An example of phospohistone-H3 immunolabeling to compliment Figure 1G.

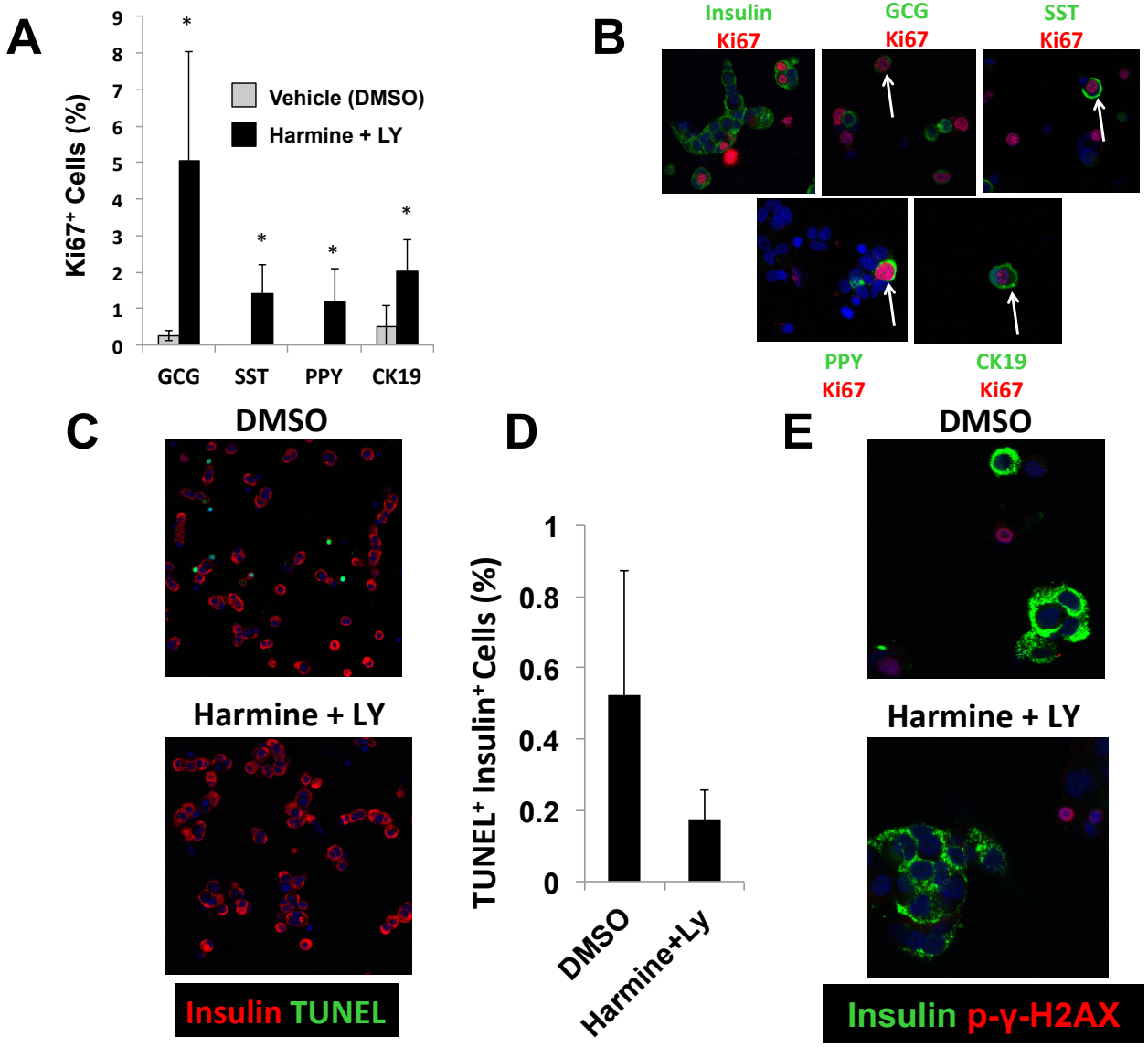

Figure S3. (Related to Fig. 1) Cell Specificity and Toxicity in Human Islets Treated with Harmine and LY364947. A. Effects of 96 hours of harmine (10 μM) alone or in combination with LY364947 (5 μM) on Ki67 immunolabeling of dispersed human alpha, delta, PP and ductal cells. Bars indicate mean ± SEM. *indicates $p<0.05$ for harmine LY combination vs. control. **B.** Examples of Ki67 labeling in each cell type. **C.** and **D.** Cell death as assessed using TUNEL labeling in dispersed human islets treated with vehicle (DMSO) or the harmine-LY364947 combination. Note that there is visible TUNEL labeling in the controls, but this, if anything, is reduced in the harmine-LY-treated cells. **E.** Phospho-gamma-H2AX immunolabeling for DNA damage responses in harmine-LY-treated dispersed human islets. There is no evidence of DNA damage.

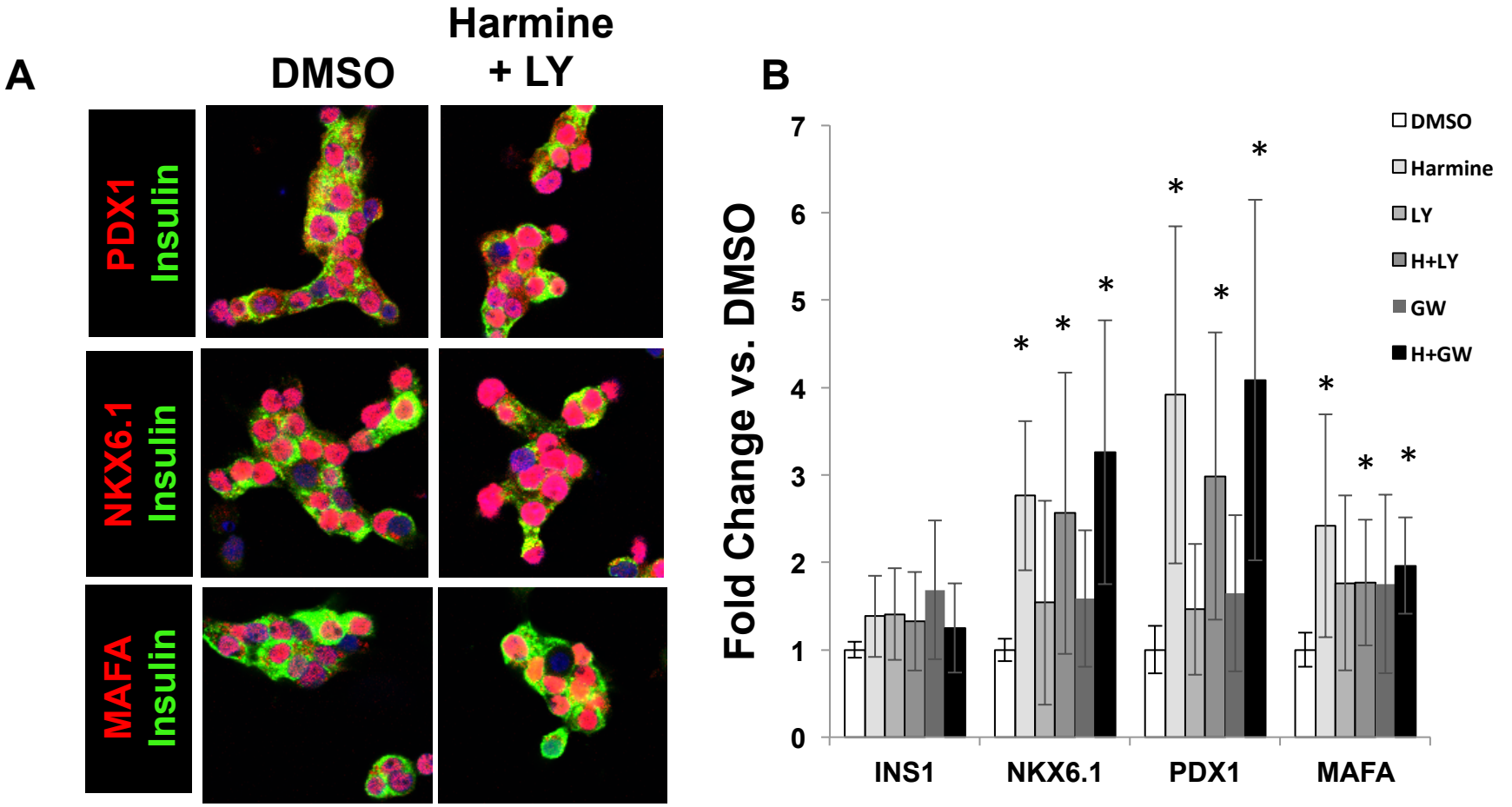

Figure S4. (**Related to Fig. 4**) **The Effects of Harmine Alone And In Combination with TGFβ Inhibitors On Beta Cell Transcription Factors in Dispersed Human Islets.** **A.** Laser confocal images of dispersed normal human islet cells immunolabeled for insulin and PDX1, NKX6.1 or MAFA following treatment with vehicle or harmine plus LY364947 for 96 hours. Compare to Figure 2C which shows similar findings using lower laser intensity. **B.** Quantitative PCR for insulin, NKX6.1, PDX1 and MAFA on dispersed human islets from six donors with Type 2 diabetes treated with vehicle or harmine or harmine plus GW788388 or harmine plus LY364947 for 96 hours. These data extend those shown in Figure 2F with harmine plus ALK5. Bars indicate mean ± SEM. Asterisks indicate $p<0.05$.

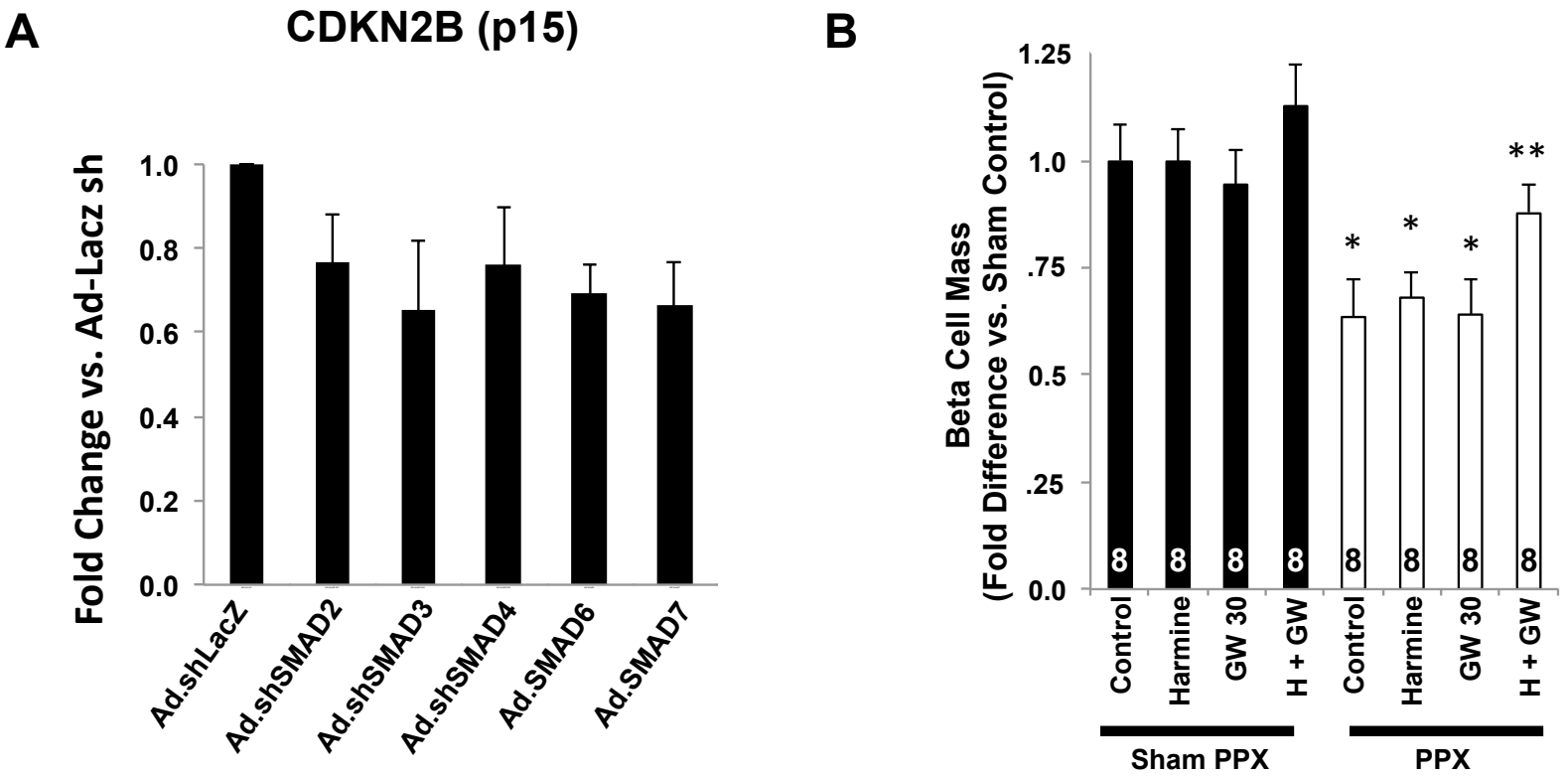

Figure S5. **(Related to Figs 4 and 6)** **A.** Effect of Silencing R-SMADs 2,3 and 4, of Overexpressing I-SMADs 6 and 7 on Expression of *CDKN2B* Encoding $p15^{INK4}$. Expression of *CDKN2B* encoding $p15^{INK4b}$ following treatment of dispersed human islets with adenoviruses expressing the shRNAs shown on the x-axis. As for *CDKN1A* and *CDKN1C* shown in Figure 4C,D, C*DKN2B* declined, but unlike *CDKN1A* and *CDKN1C,* these changes did not achieve statistical significance. Bars indicate mean ± SEM. Five different human islet donors were studied. **B.** Effect of Treatment With Control Vehicle (DMSO), Harmine, GW788388 And the Combination On Beta Cell Mass Following Real or Sham Partial Pancreatectomy (PPX) Expressed as Fold Changes Compared to Control, Sham PPX Animals. These data are the same as those shown in Figure 6C, but are normalized to the sham PPX vehicle treated control. They illustrate that the harmine-GW788388 combination administered intraperitoneally for seven days almost completely restores beta cell mass to normal following 60% pancreatectomy. They also suggest that combined harmine-GW788388 treatment may even increase beta cell mass on normal mice. Bars indicate mean ± SEM. There were eight mice in each group. *indicates beta cell mass is lower than in the sham group at $p<0.05$. **indicates that beta cell mass in the harmine+GW788388-treated PPX animals exceeded that in the three other PPX groups at $p<0.05$. It is also not significantly different from the sham (normal) beta cell mass in the four black bars.

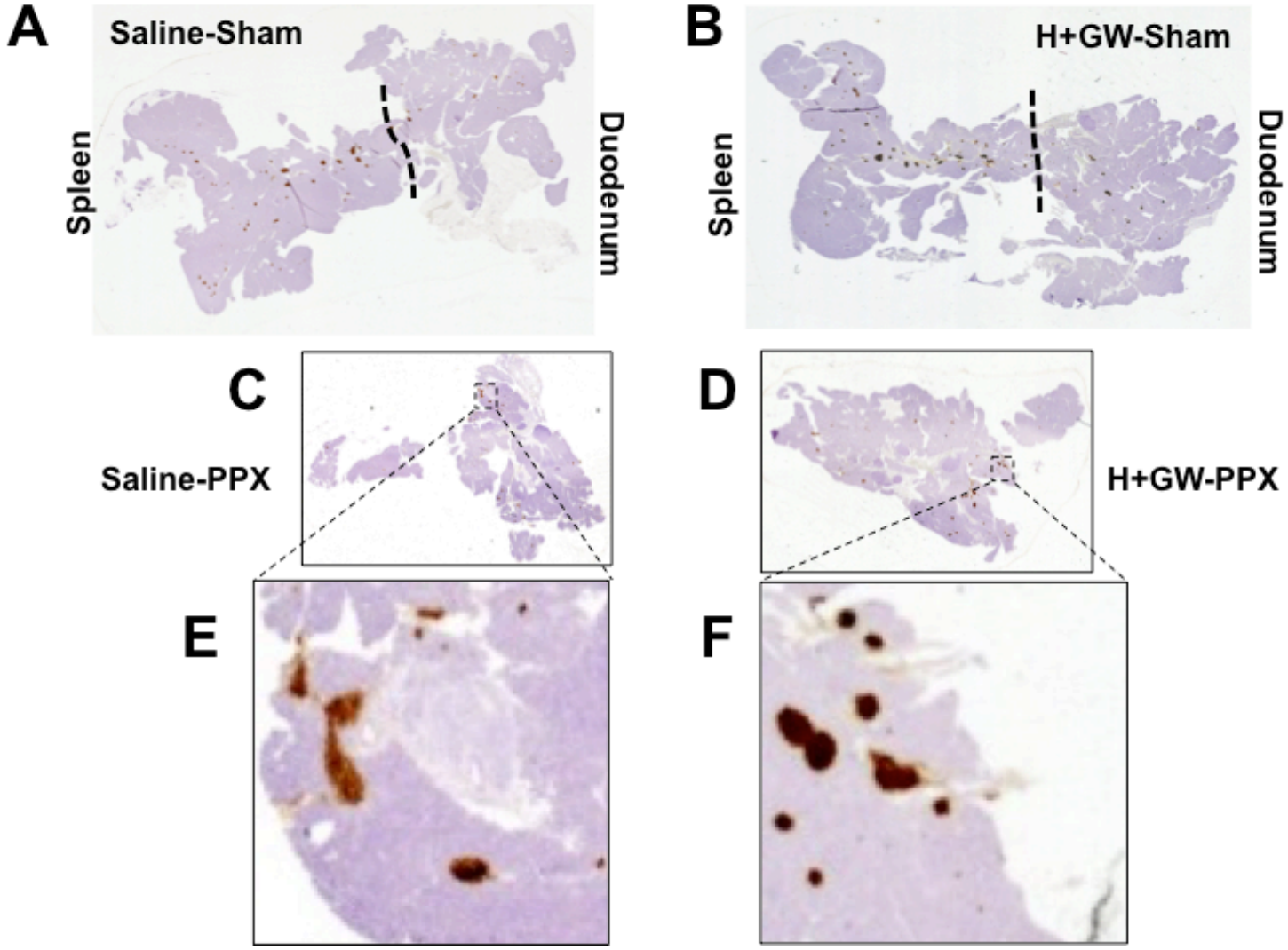

Figure S6. (Related to Fig. 1) The Effects of 60% Partial Pancreatectomy (PPX) on Beta Cell Mass In Control Mice and Mice Treated with the Harmine-GW788388 Combination. **A** and **B** illustrate low power whole pancreas sections from mice undergoing sham PPX treated for seven days with saline or the harmine-TGFβ combination as described in Results and Methods. The dotted lines indicate the approximate location of pancreas transection employed in the 60% partial pancreatectomy model. **C** and **D** illustrate low power views of pancreas remnants seven days following PPX, with or without the harmine-TGFβ combination. Background staining is with hematoxuylin, and islets are immunoperoxidase-labeled for insulin in brown. **E** and **F** are higher power magnifications of the dotted-lined boxes in C and D, illustrating insulin immunolabeling of islets within the pancreas remnants. Quantitation of beta cell mass is depicted in Fig. 6C and Supplemental Fig. 6. All images were collected using a Plustek OpticFilm 7600i scanner.

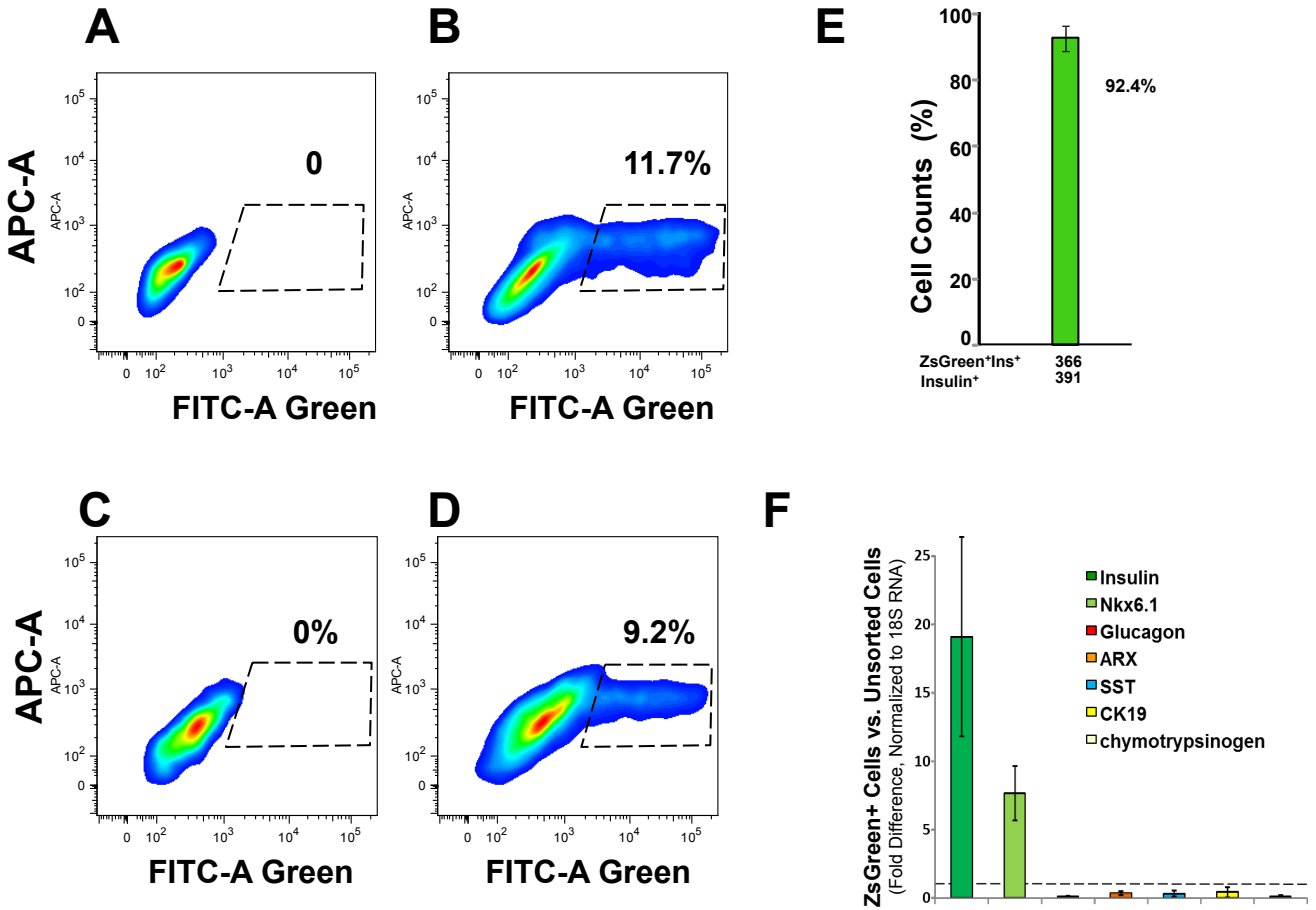

Figure S7. (Related to STAR Methods) Strategy for FACS-Sorting and Validating The Identity of Ad.RIP-ZsGreen-Sorted Human Beta Cells. Briefly, human islets are dispersed to single cells and transduced with 250 moi of a control (Ad.Cre) or Ad.RIP1-ZsGreen virus for two hours, allowed to recover and express for 72-96 hours and then are re-dispersed and sorted by flow cytometry, all as described in Methods and reference 11. **A** and **B** are derived from one normal human islet donor and **C** and **D** from a second normal donor. APC-A (650-660 nm) detects autofluorescence, and FITC-A (495-519 nm) is optimal for ZsGreen detection. In A and C, cells are transduced with a control virus and in B and D with the Ad.RIP-ZsGreen virus. Note the appearance of a large new population of ZsGreen + cells in both B and D that are not present in A and C. The dotted lines in A-D represent the gates used for sorting ZsGreen+ cells. In A-D, the % values refer to the percent of live, single ZsGreen$^+$ cells as a function of total cells. **E** shows the quantitation of insulin+ cells as a function of FACS-sorted ZsGreen+ cells. Note that 366 of 391 (92.4%) ZsGreen$^+$ cells immunolabeled for insulin. These figures represent the results of seven human islet preparations. **F** shows the ratio of endocrine, ductal, and exocrine RNAs, assessed by qPCR, in the ZsGreen$^+$ population as compared to the same mRNAs in unsorted islets from the same donors, which are normalized to a value of 1. The ZsGreen$^+$ cells are highly enriched for insulin- ~(20 fold) and Nkx6.1- (~8-fold) expressing cells, and depleted for glucagon-, Arx-, somatostatin-, cytlokeratin-19-, and chymotrypsin-expressing cells.