Supplemental Figure 1. Pdx1 levels remain constant during mitosis. The average intensities of Pdx1 at different cell cycle stages are plotted. G2, n = 46 cells; prophase, n= 23 cells; prometaphase/metaphase, n = 19 cells; anaphase n = 14 cells; telophase/early G1, n = 18 cells. The significance level after Bonferroni correction was p = 0.0125. The difference between G2 and anaphase has p= 0.0001. All the other groups have p values >0.0125.

Supplemental Figure 2. FACS analysis of other essential transcription factors in asynchronous INS1 cells. INS-1 cells immunolabeled for FoxM1(A), MafA (B), NKX6.1 (C) and counterstained with DAPI were evaluated by FACS. The protein level of Nkx6.1 (C) and FoxM1 (A) in cells at G1 (100K) and G2 (200K) showed a broader range, but had a more narrow range in S phase cells (between 100K and 200K). MafA (B) levels followed a similar trend but were less obvious.

Supplemental Figure 3. INS-1 cells with high Pdx1 levels fail to enter S/G2. Confocal images of immunolabeled INS-1 cells showing Pdx1 (purple), Aurora B (red), F-actin(green), and DAPI (blue) at different time points during cell cycle synchronization. A. Unsynchronized INS-1 cells have heterogenous Aurora B expression patterns. White arrow indicates a cell with very low Aurora B expression. Yellow arrow indicates a cell with high Aurora B. Green arrow indicates Aurora B at the midbody during cytokinesis. The majority of the other cells are Aurora B-negative. B. Immediately after releasing cells from arrest, almost all cells are Aurora-negative. C. 4 hr after releasing cells from arrest, most of cells have different levels of Aurora B expression. C. 12 hr after releasing from arrest, most cells have high Aurora B expression. White arrow indicates a cell with condensed chromosomes. Cells in the white boxes are Aurora B-negative and express Pdx1 at high levels.

Supplemental Figure 4. Pdx1 overexpression promotes cell cycle progression in RPE1 Cells. FACS analysis of cell-cycle progression in NLS-mCherry expressing RPE1 cells (A) and mCherry-Pdx1 expressing cells (B). Black lines represent the original experimental data. Purple lines and colored areas are results from Flow Jo cell cycle analysis: Light blue areas represent G0/G1, yellow areas represent S, green areas represent G2/M. Purple lines represent the overall fitted curve. The percentages showed in each plot are the ratio out of G0/G1 + S + G2/M