Aging and insulin secretion in young β-cells phenocopies aged β-cell metabolism and insulin secretion

### Question: What happens during aging to regulate insulin secretion?

Cyclin-dependent kinases (CDKs) are at the intersection of aging and metabolism. Aging promotes the expression of senescence factors, which inhibit CDK activity. CDKs activate the cell cycle.

1. **Q1.** Attenuation of cyclin-dependent kinases in young β-cells phenocopies aged β-cells.
2. **Q2.** Is CDK2 required for this regulation?

#### Figure 1A. The percentage of adults with diabetes increases with age. Data from the Center for Disease Control and Prevention National Diabetes Statistics Report, 2017. Error bars, 95% confidence interval.

#### Figure 1B. Architecture of the pancreas. Glucose is sensed in the pancreas by the islets of Langerhans. Each islet contains specialized cell types that sense nutrients and release hormones that signal to the β-cell to fine-tune insulin secretion.

#### Figure 1C. Glucose stimulates insulin secretion by its metabolism in the β-cell. Glucose metabolism increases the ATP/ADP ratio, which closes the K_{ATP} channel and depolarizes the membrane. This results in an influx in calcium, which triggers insulin granule secretion. Insulin promotes glucose uptake out of the bloodstream by other tissues.

#### Figure 2. GSIS is decreased in aged humans, but increased in aged mouse islets.

#### Figure 2A. Functional islet assays. Islets are isolated from mouse or human pancreas, then stimulated with either 2 mM glucose (2G) or 17 mM glucose (17G). Insulin released by the islets into the media was measured.

#### Figure 2B. Human islets decline in function with age. Insulin secretion reported as % islet insulin content. n=31.

#### Figure 2C. Mouse islets improve in function with age. Young, 103 islets from 2 young mice (4-6 months old). Aged, 122 islets from 5 aged mice (18-30 months old).

#### Figure 3. Mitochondrial function is reduced in aged humans and aged mouse islets.

#### Figure 3A. Measuring mitochondrial function. Using a novel NAD(P)H imaging platform we interrogated mitochondrial function in human and mouse islets.

#### Figure 3B. NAD(P)H Fluorescence Lifetime Imaging demonstrates a age-dependent decline in mitochondrial NAD(P)H metabolism in both human and mouse islets. Human islets, n = 20 islets from 10 human islet donors. Mouse islets, n = 10 islets per condition.

#### Figure 4. Experimental Procedures

#### Figure 4A. Mouse models used to answer Q1. We inducibly deleted Cdk2 specifically from mouse β-cells (Cdk2-/-KO). After islet isolation we compared mitochondrial function and insulin secretion in Control and Cdk2-/-KO islets.

#### Figure 4B. Mouse models used to answer Q2. We compared lean mice and obese mice to understand the influence of obesity on islet CDK1 function. After isolation islets were either treated with Cdk1 inhibitor (Cdk1i) or left untreated. Mitochondrial function and insulin secretion assays were performed following the drug treatment.

#### Figure 5. CDK2 inhibition reduces mitochondrial function, and enhances GSIS.

#### Figure 5A. Mitochondrial function is inhibited by CDK2 deletion (Cdk2-/-KO). Islets isolated from Cdk2-/-KO mice showed reduced mitochondrial activity, similar to aged mice. n = 10 islets per mouse from 3 mice per condition.

#### Figure 5B. Insulin secretion is increased by CDK2 deletion. Despite the reduction in mitochondrial activity (Fig. 5A), CDK2 deletion enhanced GSIS, similar to observations in aged mice. n = 5 mice per condition.

#### Figure 6. CDK1 inhibition reduces mitochondrial function, and enhances GSIS.

#### Figure 6A. Mitochondrial function is activated by obesity and inhibited by CDK1 inhibitor (CDK1i). Islets isolated from obese (ob) mice showed a higher dependence on CDK1-activated mitochondrial function than lean counterparts. n = 10 islets per mouse from 5 mice per condition.

#### Figure 6B. Insulin secretion is increased by obesity and further increased by CDK1 inhibitor. Despite the reduction in mitochondrial activity (Fig. 6A), CDK1 inhibition enhanced GSIS in both lean and ob islets. Again, ob islets were more sensitive to this inhibition. n = 3 mice per condition.

#### Conclusions

1. **Seniors are disproportionately affected by Type 2 Diabetes.** Because age is a major risk factor for disease development, understanding how β-cells age is critical to developing preventative therapies.
2. **Human islets exhibit an age-dependent decline in insulin secretion, but aged mouse islets improve with age.** We can leverage the differences between species to understand how mouse β-cells successfully age.
3. **Mitochondrial function declines in both aged human and mouse islets.** This decline is conserved across species.
4. **Restricting CDK signaling in young mice islets reproduces the results observed in aged islets. Deleting CDK2 from β-cells or inhibiting CDK1 with a drug has a similar phenotype to ("phenocopies") aged mice.
5. **Obesity renders β-cells more sensitive to CDK1 inhibition.** Obesity stimulates β-cell division by upregulating CDK1 signaling. When CDK1 is inhibited, obese islets are thus more sensitive than lean islets.