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Supplemental Information

β Cell Aging Markers Have Heterogeneous Distribution and Are Induced by Insulin Resistance

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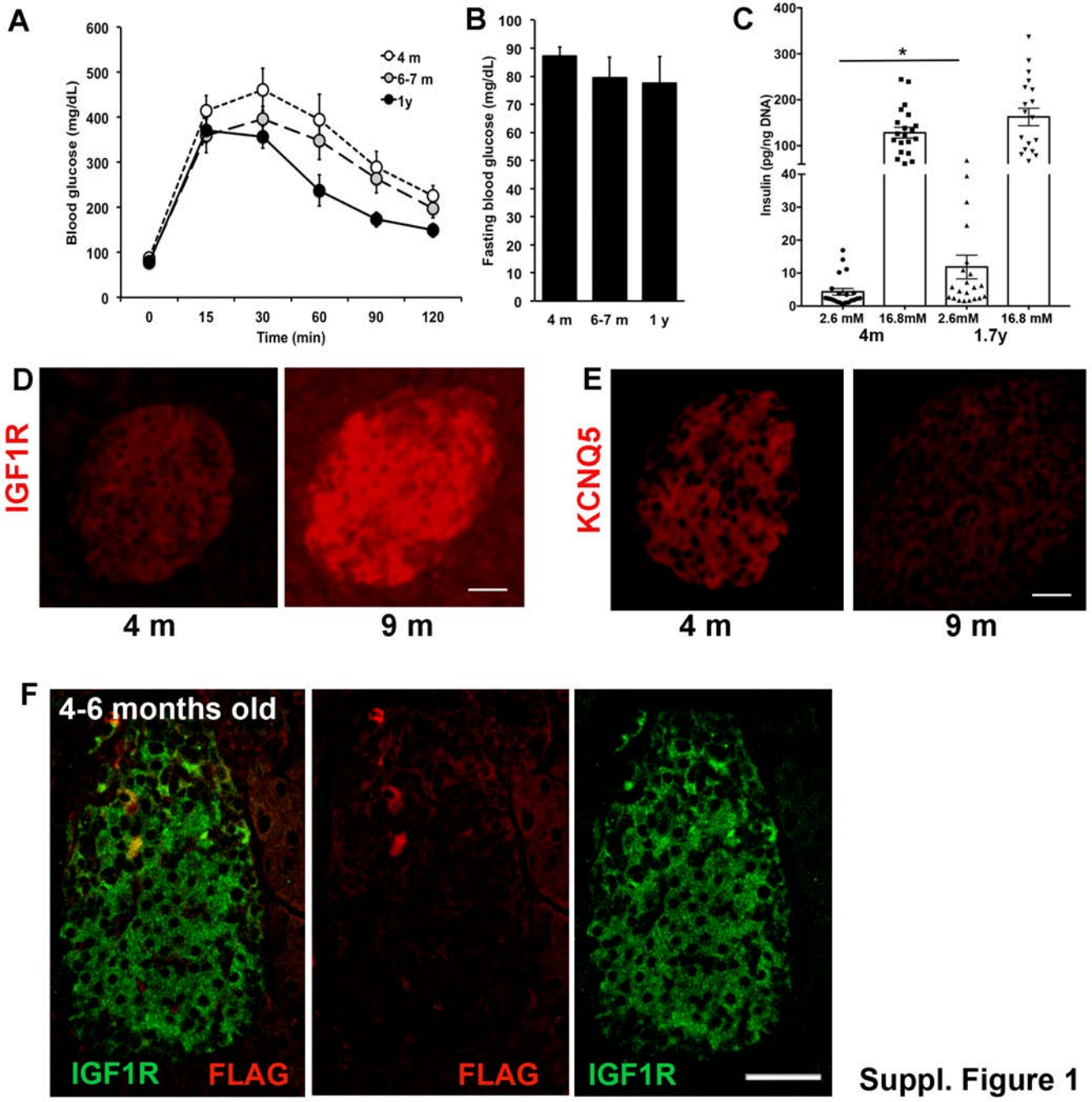
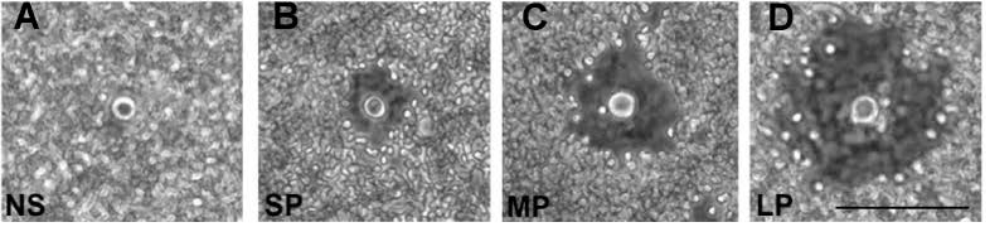
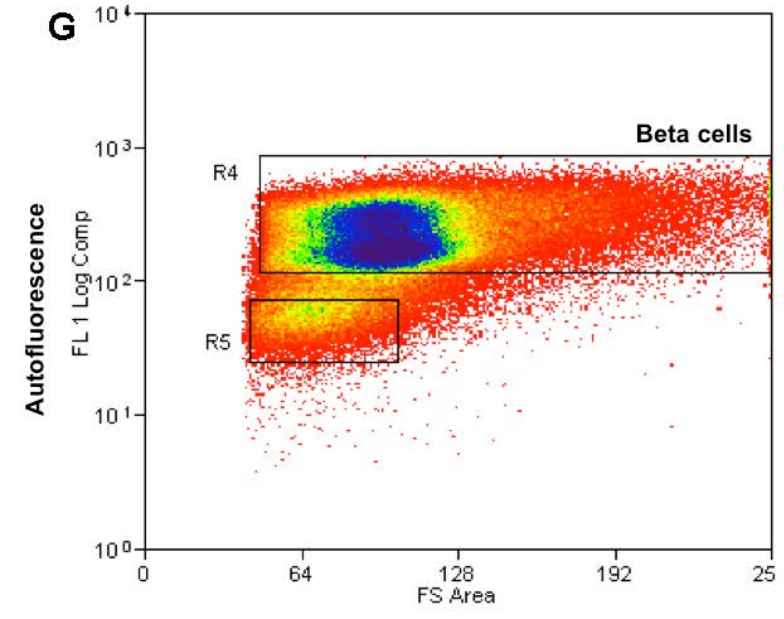
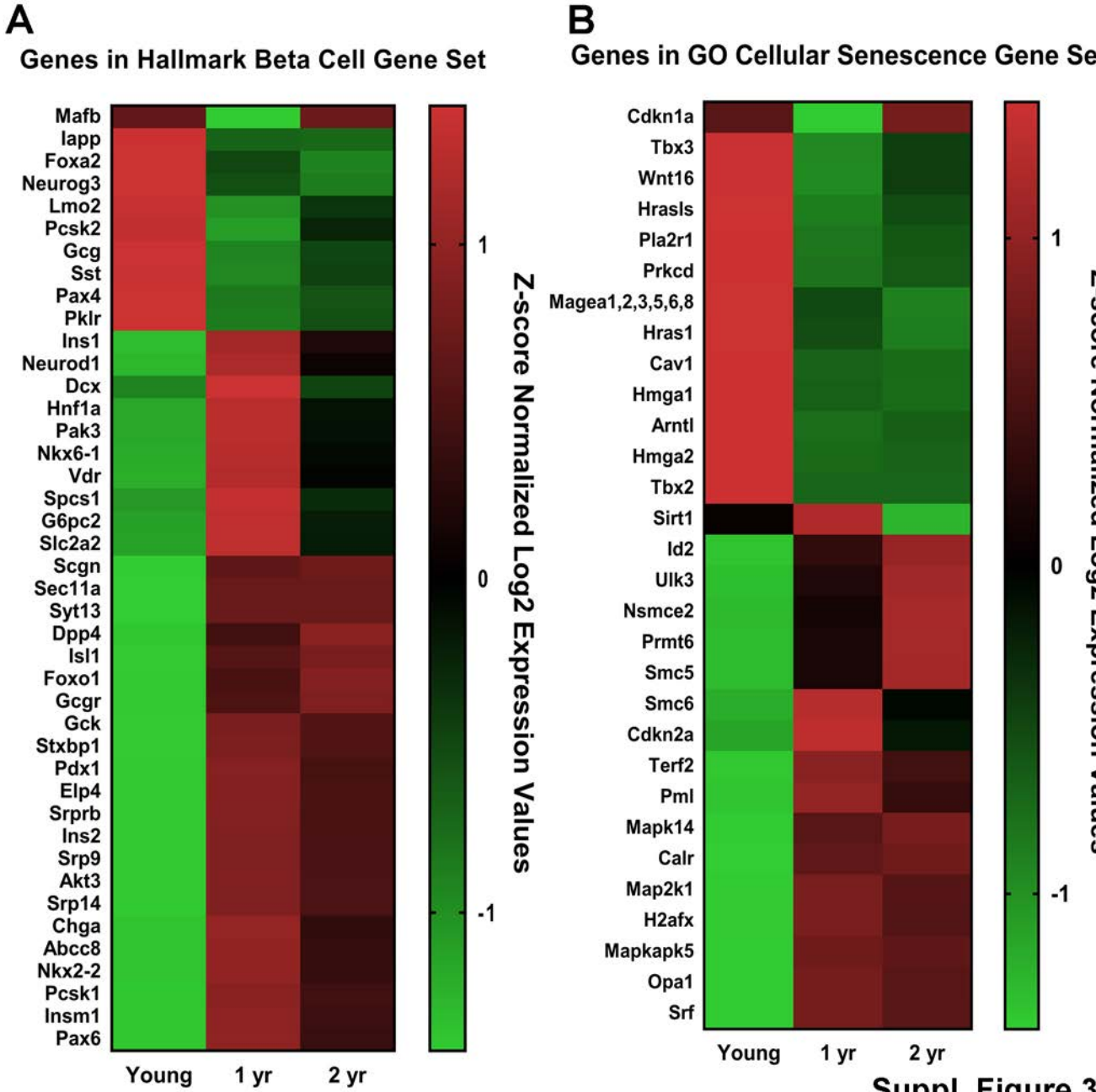


Suppl. Figure 1





Suppl. Figure 2



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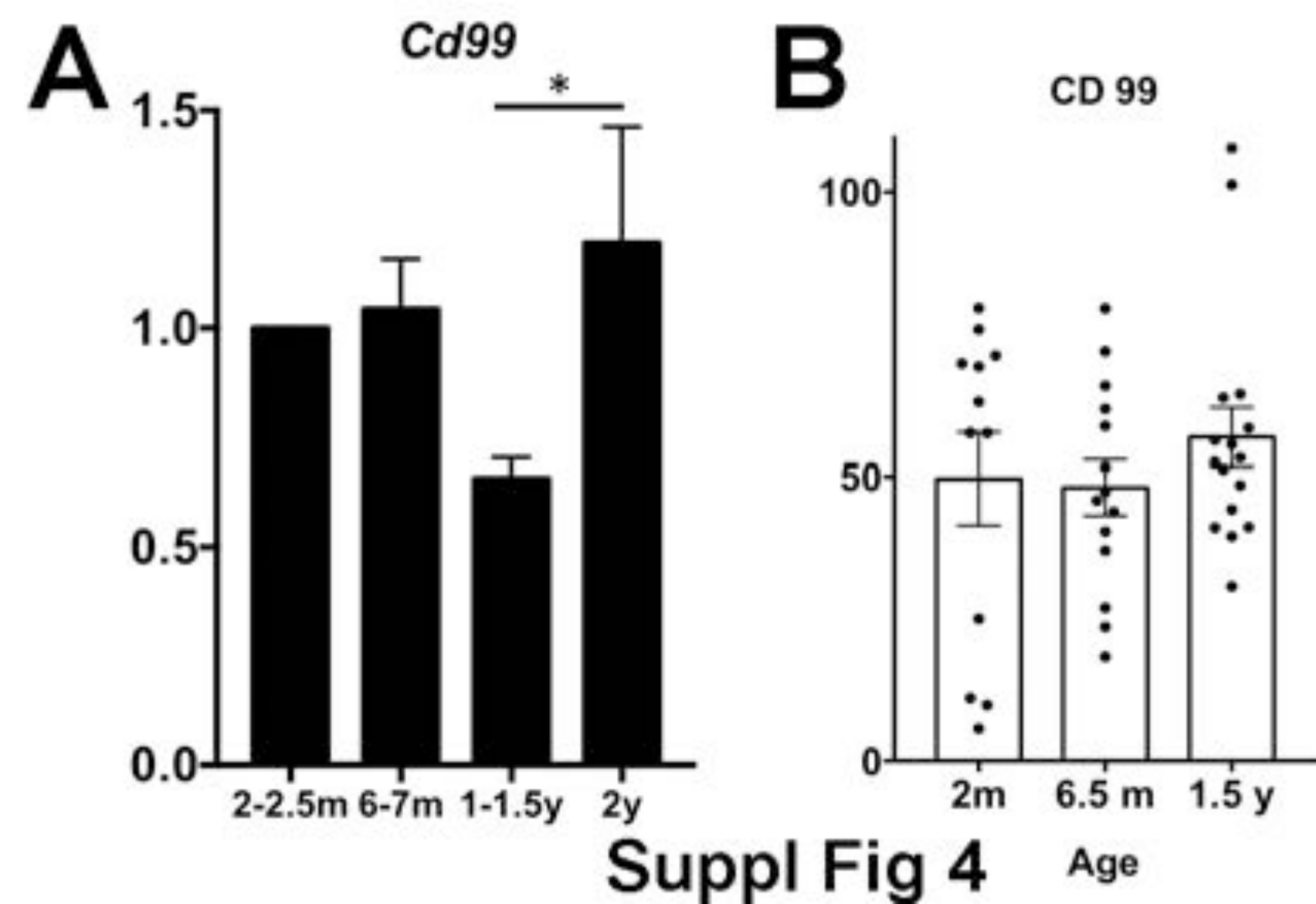

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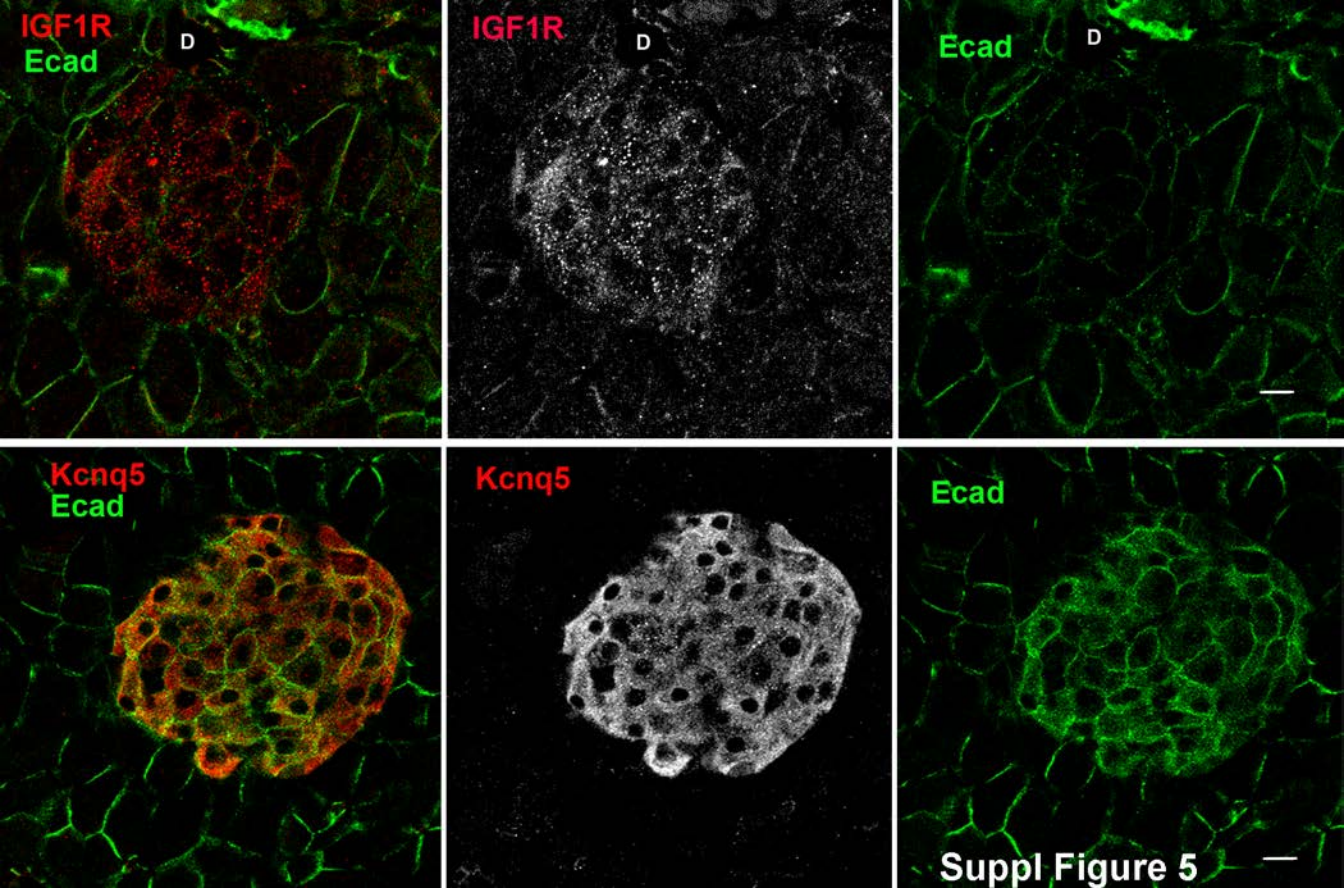
IGF1R
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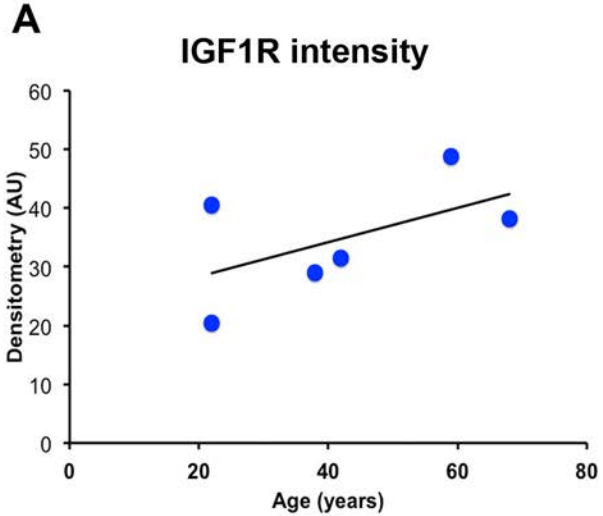
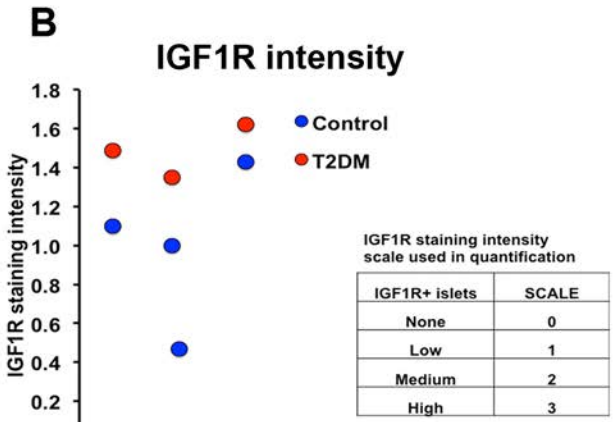
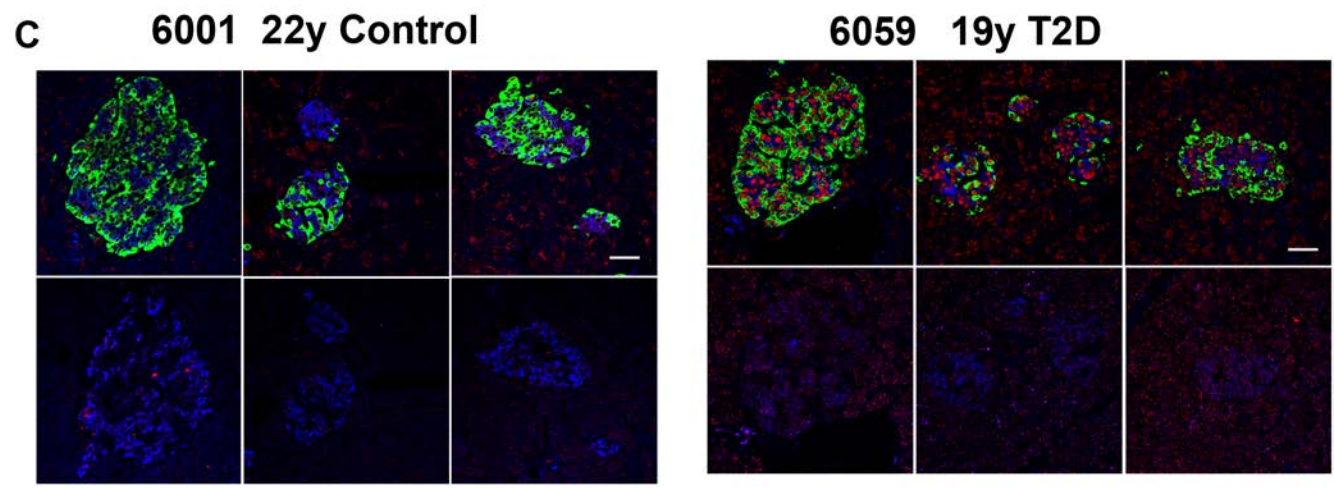
Suppl Figure 5

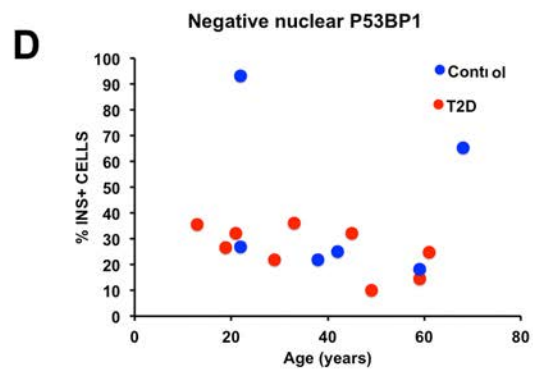


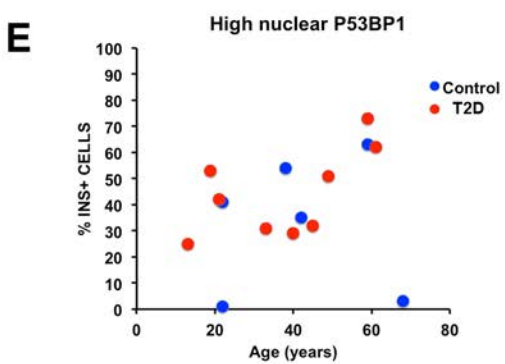


IGF1R staining intensity scale used in quantification

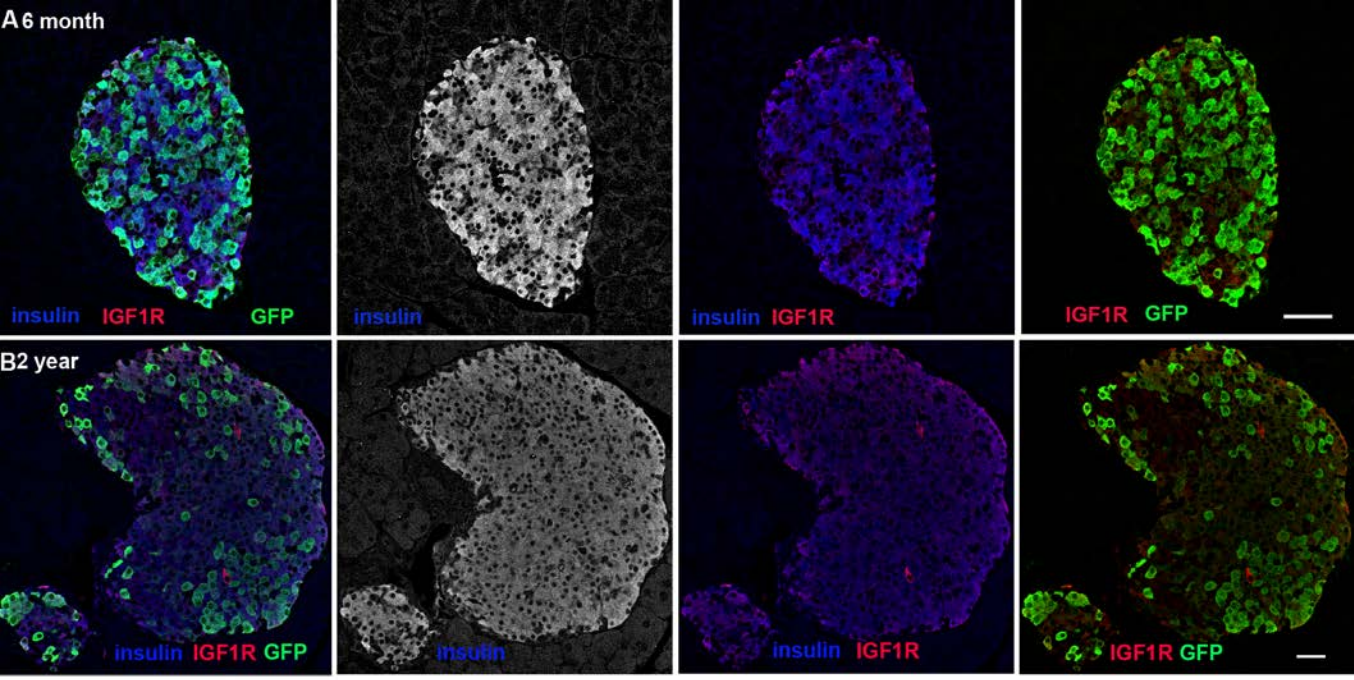
IGF1R+ islets	SCALE
None	0
Low	1
Medium	2
High	3

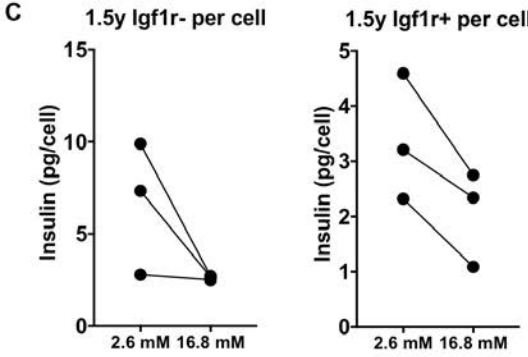






Suppl. Figure 6





Suppl. Figure 7

SUPPLEMENTAL INFORMATION

Supplementary Figure 1. Changes of β-cell aging markers occur even in aged mice that remain normoglycemic. Related to Figures 1 and 3. INK-ATTAC mice at 1y show neither a decline in glucose tolerance after IPGGT (**A**) nor fasting hyperglycemia (**B**). Yet with age there is an increase in basal insulin secretion (**C**). Data presented for each of the triplicate samples (10 islets) of 7 individual mice/age. Additionally with age IGF1R protein levels increased (**D**) and KCNQ5 protein decreased (**E**). n=4 animals/age. **F**. Co-localization of IGF1R and p16 reporter FLAG in a subset of cells from 4-6 m INK-ATTAC mice. Data are Means ± SEM. Magnification bar=25 μm (D, E); 50 μm (F).

Supplementary Figure 2. Individual β-cell insulin secretion and β-cell FACS sorting criteria. Related to Figure 1 and STAR methods. In reverse hemolytic plaque assay (RHPA), the immunoplaque area is directly proportional to the amount of insulin secreted by individual β cells. Representative pictures of non-secreting (**A**), small plaques (**B**), medium plaques (**C**) or large plaques (**D**); this image has been previously published in (Aguayo-Mazzucato et al., 2011). Magnification bar= 100 μm Insulin secretion is comparable using static incubation (insulin pg/ngDNA) **(E**) or the reverse hemolytic plaque assay (secretion index=% secreting cells X plaque area) (**F**). **G**. FACS sorting criteria for a purified fraction of β cells based on autofluorescence (King et al., 2007). Data are Means ± SEM.

Supplementary Figure 3. Age-induced changes in β-cell and senescence gene sets. Related to Figure 2C. Heatmaps of gene expression from microarray data of purified β-cells from young, 1y and 2y old mice showing changes in expression of Hallmark β cell (A) and GO Cellular senescence (B) gene sets. Comparison of young to 1 y shows increased maturation of β-cell identity whereas comparison of 1y and 2 y show increase in cellular senescent genes. These data show that some characteristic β-cell genes are turned off with aging however, the overall gene expression profile is very different between

young and 2 year old supporting the presence of different phenotypes at different life stages of a β-cell.

Supplementary Figure 4. ***CD99*** **mRNA and protein timecourse. Related to Figure 2F.** Timecourse expression of *CD99* mRNA (**A**) and quantification of protein expression by densitometry(**B**). Same samples as used in Figure 2F and 3A. At 2m there are two subpopulations, a high and low; the low disappears with age. Data are Means ± SEM.

Supplementary Figure 5. Plasma membrane expression of aging markers. Related to Figure 3. IGF1R and KCNQ5 colocalize with cell membrane marker E-Cadherin. Plasma membrane staining of IGF1R is more clearly seen in ducts (D) and acinar cells, which have lower levels of cytoplasmic expression than β cells. Merged and then single channels shown. Magnification bar=10 μm

Supplementary Figure 6. Expression of β-cell aging markers in human β-cells and changes induced by T2D. Related to Figures 3F and 6. β-cells of human donors express higher levels of IGF1R protein with age (**A**) and with T2D (**B**) protein as seen by immunostaining. Magnification bar=50 μm **C**. The presence of T2D in young donors appears to induce P53BP1 and IGF1R expression. Quantification of β-cells that were negative (**D**) or highly positive (**E**) for nuclear P53BP1 in pancreas from donors with and without T2D over a range of ages. Details of the human donors are given in **Key Resources.** Data are values for individual donors.

Supplementary Figure 7. Expression of IGF1R in β-cells from MIP-GFP mice and insulin secretion from IGF1R positive and negative β-cells obtained from old mice. Related to Figures 3I and 4C. Representative pictures of insulin, IGF1R and GFP co-expression from 6 m (**A**) and 2 y (**B**) MIP:GFP mice showing the decline of GFP expression with age. Merged and separated channels. Magnification bar=50 μm. GFP antibody had been optimized to show differential

expression whereas that of insulin was not. Even GFP^{low} were stained for insulin. **C**. Both IGF1R+ and IGF1R- β-cells from 1.5 y C57Bl/6J mice lack glucose-stimulated insulin secretion. n=3 independent cell preparations. Data are Means ± SEM

Supplementary Table 1. Top 550 cells surface genes differentially expressed between young and aged β cells as shown in the volcano plot (**Related to Fig. 2D**).

REFERENCES FOR SUPPLEMENTAL INFORMATION

Aguayo-Mazzucato, C., Koh, A., El Khattabi, I., Li, W.C., Toschi, E., Jermendy, A., Juhl, K., Mao, K., Weir, G.C., Sharma, A., et al. (2011). Mafa expression enhances glucose-responsive insulin secretion in neonatal rat beta cells. Diabetologia *54*, 583-593.

King, A.J., Fernandes, J.R., Hollister-Lock, J., Nienaber, C.E., Bonner-Weir, S., and Weir, G.C. (2007). Normal relationship of beta- and non-beta-cells not needed for successful islet transplantation. Diabetes *56*, 2312-2318.