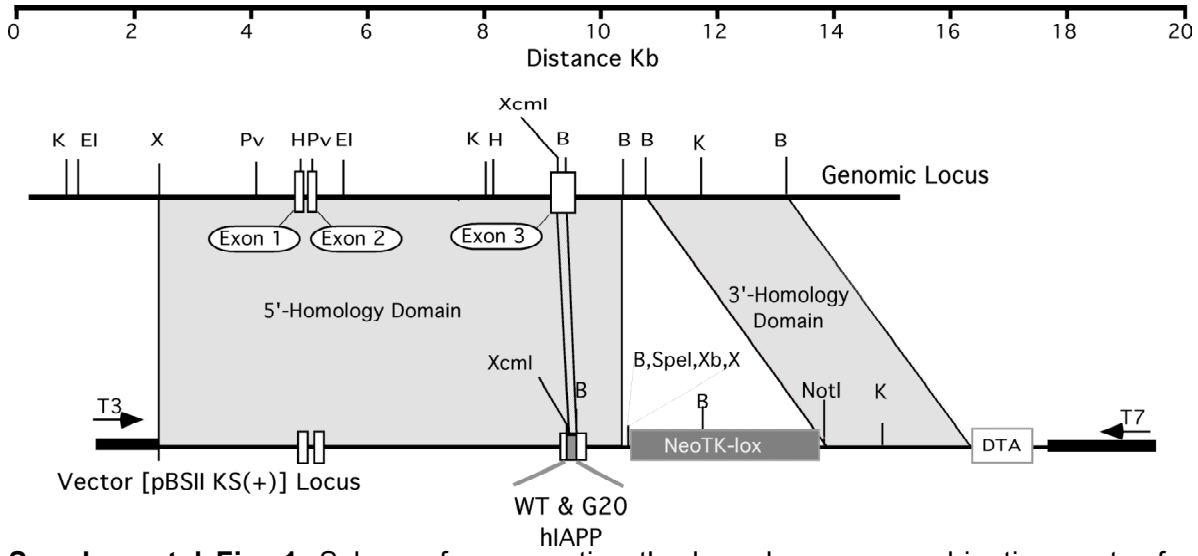
APPENDIX S1:

Generation of hIAPP WW- and GG-Knock-In (KI) Mice. All experiments with mice were approved by the Mayo Institutional Animal Care and Use Committee. We constructed two homologous recombination vectors for hIAPP^{WT} and hIAPP^{S20G} (Supplemental Figure 1) from bacteriophage λMIAP-G2 from a DBA mouse genomic library[1]. The constructs consist of four parts: **(i)** a 5'-homologous recombination domain



Supplemental Fig. 1. Scheme for generating the homologous recombination vector for generating hIAPP^{WT} and hIAPP^{S20G} knock-in mice. K= KpnI, EI= EcoRI, X=XhoI, Pv=PvuII, H=HindIII, B=BamHI, Xb=XbaI.

(5'-HRD) consisting of >7 Kbp 5'-homology region containing the mIAPP 5'-flanking DNA, promoter, exons 1 and 2, and exon 3 engineered to contain sequences encoding hIAPP^{WT} or hIAPP^{S20G} and 3'-UTR/3'-flanking DNA region that contained the polyadenylation site; **(ii)** a NeoTK-lox cassette, allowing positive selection of ES cell recombinants with neomycin and negative gancyclovir selection of NeoTK-lox-containing genomes not excised by cre recombinase[2]; **(iii)** the 3'-HRD consisting of 2.5 kb of 3'-flanking DNA from the mIAPP gene; and **(iv)** the diphtheria toxin A gene (DTA) that eliminates non-homologous recombination events[3].

The 5'-HRD was constructed by cloning the *Xho*I/*Bam*HI fragment, containing the 5'-flanking DNA, promoter and exons 1-3 of the mouse IAPP gene into pBlueScriptII(KS+). The mouse sequences in exon 3 were then replaced by substituting synthetic DNA fragments containing *Xcm*I/*Bam*HI restriction sites and the sequences from the hIAPP^{WT} and hIAPP^{S20G} genes into the *Xcm*I/*Bam*HI site of the 5'-HRD. The 7.0 kb 5'-HDR was completed by cloning a 0.8 kb *Bam*HI fragment containing the mIAPP 3'-UTR and polyadenylation site into the vector at the *Bam*HI site. Next the NeoTK LOX cassette (provided by Dr. C. Ronald Kahn, Joslin Diabetes Center, Boston, MA) was cloned downstream of the 5'-HRD. A *Not*I site was inserted at the 3'-end of NeoTK–lox cassette by excising a downstream *Sal*I/*Hind*III fragment and re-ligating the plasmid in the presence of a *Sal*I/*Not*I/*Hind*III adapter. The NeoTK LOX cassette was excised with *Xba*I/*Not*I and inserted into pBlueScriptII(KS+). The NeoTK LOX cassette

was excised with *Spe*I/*Not*I and cloned downstream of the 5'-HRD at the *Spe*II/*Not*I sites in 5'-HRD-pBlueScriptII(KS+).

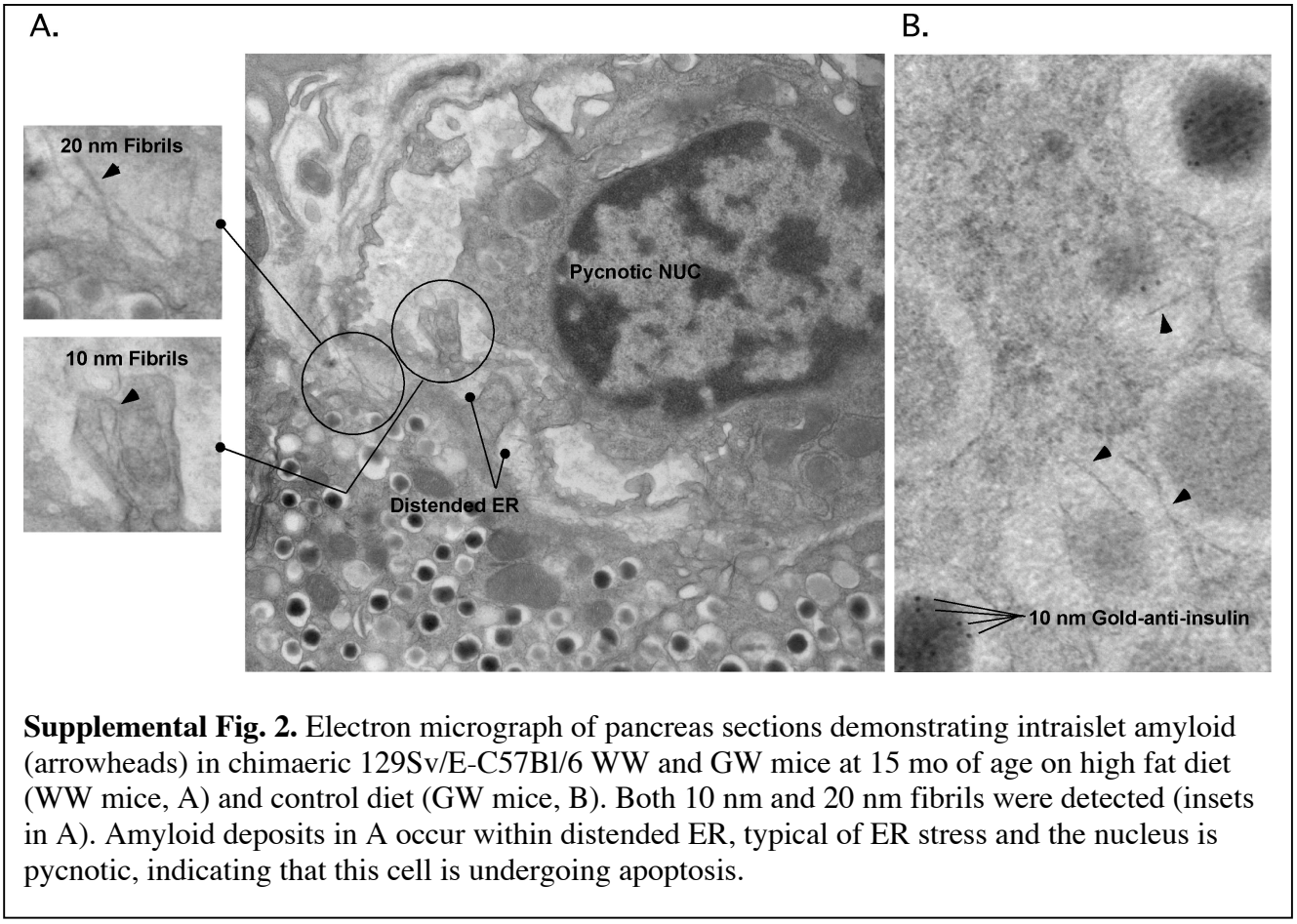
The 3'-HRD and DTA gene were cloned together into pBlueScriptII(KS+). The *Asc*I site at the 3'-end of the DTA gene was converted to a *Sac*II site by removing an *Asc*I/*Xho*I fragment and re-ligating in the presence of an *Asc*I/*Xho*I adaptor containing a *Sac*II site. The DTA gene was then excised by *SpeI*/*Sac*II digestion and inserted into *Spe*I/*Sac*II-digested pBlueScriptII(KS+). The 2.5 kb 3'-HRD *Bam*HI fragment, containing the 3'-flanking DNA from the mIAPP gene was cloned into the *Bam*HI site of this vector and the correct orientation was confirmed by sequence analysis. Subsequently, a *Not*I site was introduced upstream of the 3'-HRD by excising a *Psp*OMI/*Xho*I fragment and re-ligating in the presence of a *Psp*OMI/*Not*I/*Xho*I adapter. The 3'-HRD/DTA fragment was excised with *Not*I/*Sac*II and subcloned into the 5'-HRD/NeoTK-lox targeting vector at the *Not*I/*Sac*II site.

Both constructs were electroporated[4] into 129Sv/E ES cells and cultured on irradiated SNLH9 feeder cells[5] with G418. DNA analysis of 200 recombinant ES cell lines resulted in 2 $hIAPP^{S20G}$ and 4 $hIAPP^{WT}$ cell lines with the appropriate homologous recombination. Injection of mouse C57BL/6 blastocysts yielded agouti offspring and males were screened for the knock-in alleles by Southern analysis. Agouti males harboring the correct genotypes were then bred with C57BL/6 females and the resulting offspring were genotyped via PCR. Offspring with WM and GM genotypes were obtained, where W and M designate human and mouse wild-type IAPP alleles, respectively, and G designates the $hIAPP^{S20G}$ mutant allele. MM chimaeric animals lacking the hIAPP geneotypes were bred for controls. Mice were interbred to generate WW, GG, GW and MM animals for phenotypic characterization.

We examined the MM, WW, and GG chimaeric mice at 3 mo by oral glucose tolerance testing (OGTT). Unexpectedly, 3/8 (37.5%) of MM chimaeric animals exhibited abnormal OGTT tests with severe glucose intolerance (not shown), while 8/8 WW and 8/8 GG animals had normal OGGT profiles. Since the chimaeric MM mice are the obligatory control group, the presence of glucose intolerance in this group represented a significant confounding effect that may be due to 129Sv/E chimaerism. Indeed, two separate studies have provided evidence that the 129Sv/E strain contains modifier genes that predispose animals to the development of diabetes[6,7]. Therefore we backcrossed all our animals for 5 generations (N_5, 96.9% congenicity) against C57Bl/6 mice to achieve a consistent genetic background. All MM, WW and GW mice introduced into the study protocols were 5^{th} generation backcross animals to minimize differences in genetic background.

While the animals were being backcrossed against C57Bl/6 mice to remove the confounder genetic contribution of the 129Sv/E strain, we elected to subject a number of the WW and GW founder animals to a study on control and high fat diet as a preliminary experiment to determine whether the animals would exhibit evidence of intraislet amyloid deposts. Supplemental Figure 2 demonstrates that amyloid deposits were detectable in the islets of both WW animals maintained on high fat diet (15 mo old animals maintained on high fat diet for 12 mo) as well as GW animals maintained on a control diet at 15 mo of age.

APPENDIX S2:



Supplemental Fig. 2. Electron micrograph of pancreas sections demonstrating intraislet amyloid (arrowheads) in chimaeric 129Sv/E-C57Bl/6 WW and GW mice at 15 mo of age on high fat diet (WW mice, A) and control diet (GW mice, B). Both 10 nm and 20 nm fibrils were detected (insets in A). Amyloid deposits in A occur within distended ER, typical of ER stress and the nucleus is pycnotic, indicating that this cell is undergoing apoptosis.

References:

1. Ekawa K, Nishi M, Ohagi S, et al. Cloning of mouse islet amyloid polypeptide gene and characterization of its promoter. J Mol Endocrinol 1997; 19: 79-86.
2. Araki K, Imaizumi T, Sekimoto T, et al. Exchangeable gene trap using the Cre/mutated lox system. Cell Mol Biol 1999; 45: 737-750.
3. Yanagawa Y, Kobayashi T, Ohnishi M, et al. Enrichment and efficient screening of ES cells containing a targeted mutation: the use of DT-A gene with the polyadenylation signal as a negative selection maker. Transgenic Res 1999; 8: 215-221.
4. Steeghs K, Oerlemans F, Wieringa B. Mice deficient in ubiquitous mitochondrial creatine kinase are viable and fertile. Biochim Biophys Acta 1995; 1230: 130-138.
5. van Deursen J, Schepens J, Peters W, et al. Genetic variability of the murine creatine kinase B gene locus and related pseudogenes in different inbred strains of mice. Genomics 1992; 12: 340-349.

6. Terauchi Y, Matsui J, Suzuki R, et al. Impact of genetic background and ablation of insulin receptor substrate (IRS)-3 on IRS-2 knock-out mice. J Biol Chem 2003; 278: 14284-14290.
7. Suzuki R, Tobe K, Terauchi Y, et al. Pdx1 expression in Irs2-deficient mouse beta-cells is regulated in a strain-dependent manner. J Biol Chem 2003; 278: 43691-43698.