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Supplemental information

Sex-biased islet β cell dysfunction is caused by the MODY MAFA S64F variant by inducing premature aging and senescence in males

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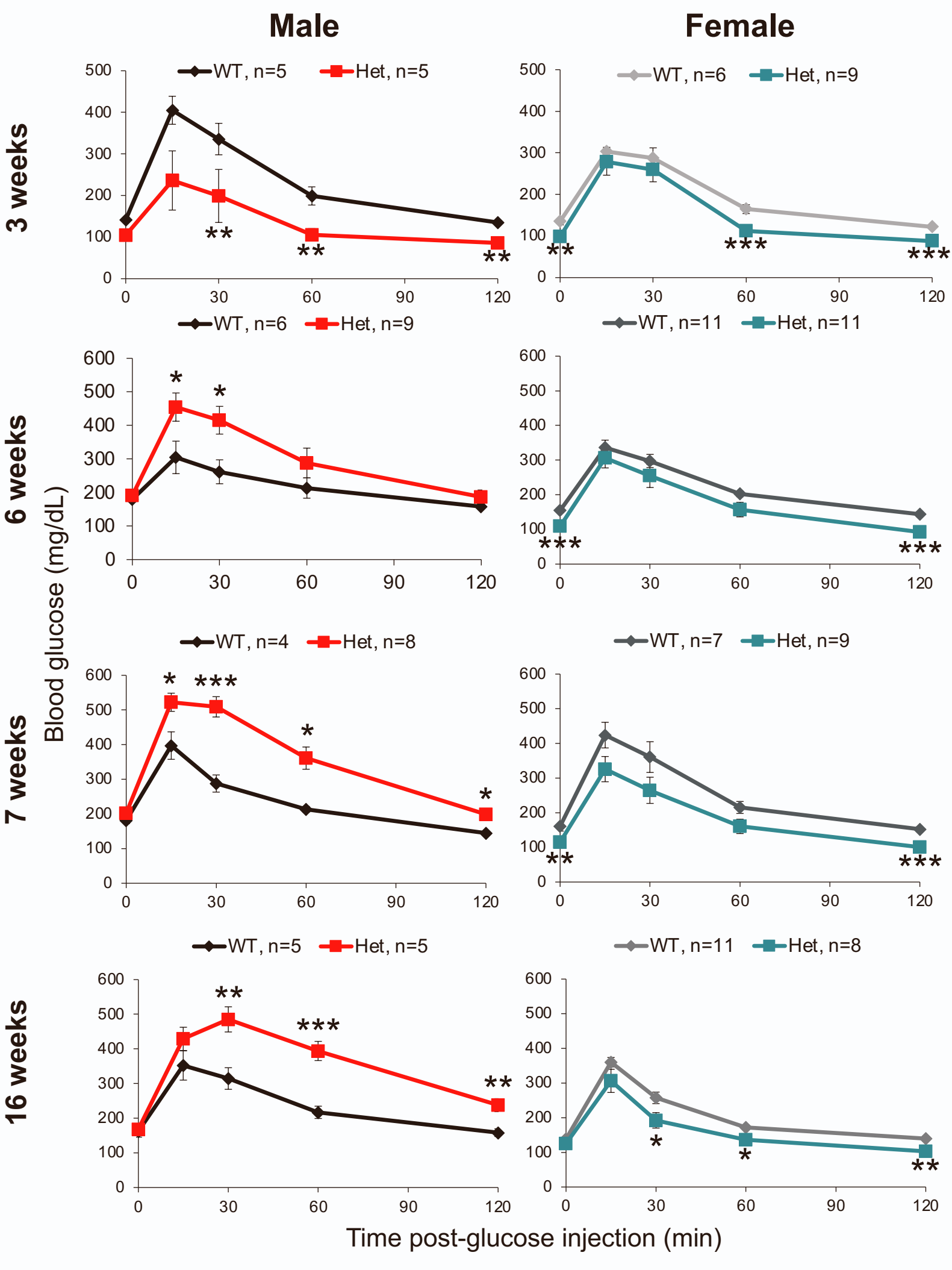
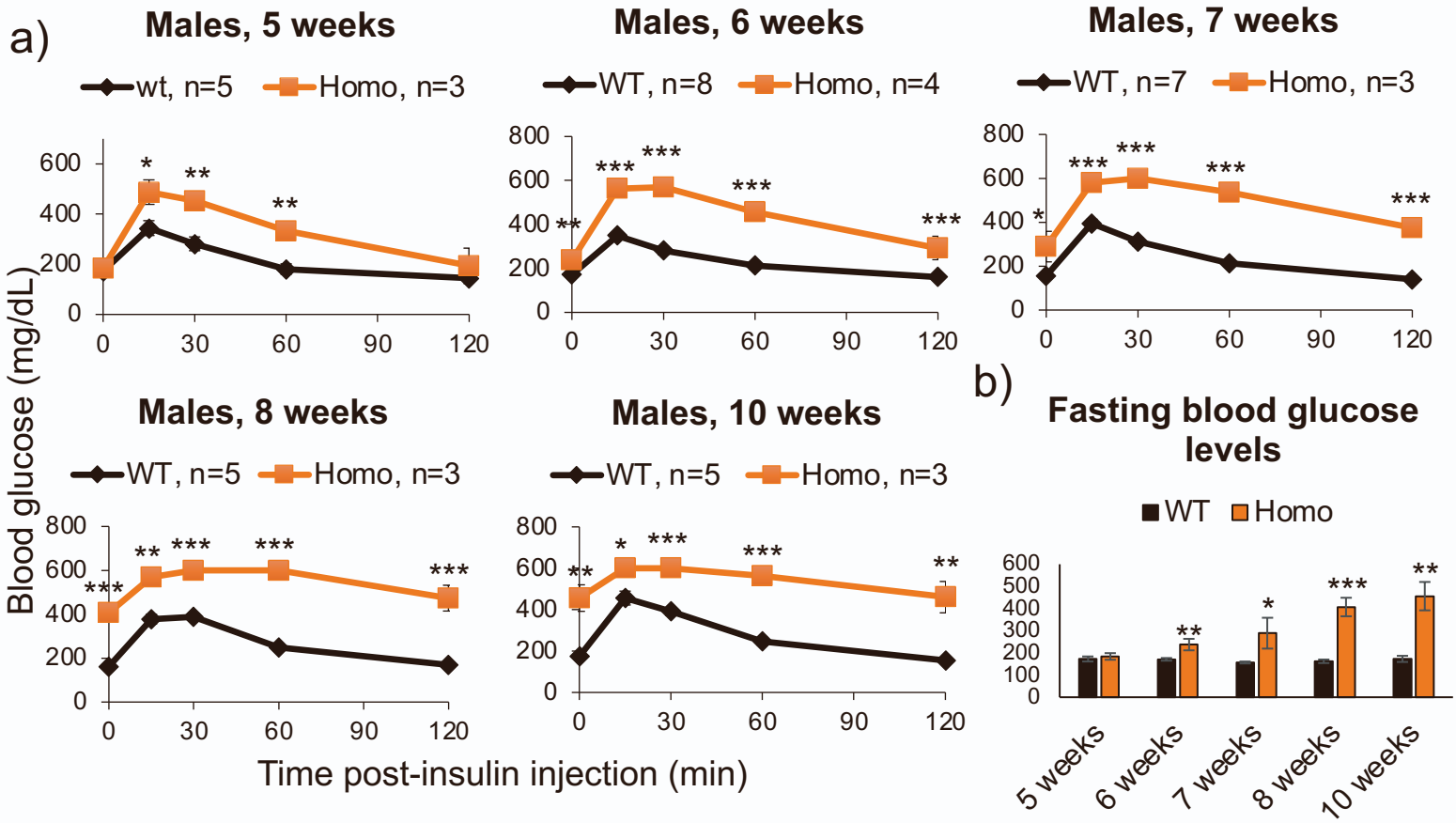

Male
Female
3 weeks
6 weeks
7 weeks
16 weeks
WT, n=5
Het, n=5
WT, n=6
Het, n=9
WT, n=6
Het, n=9
WT, n=11
Het, n=11
WT, n=4
Het, n=8
WT, n=7
Het, n=9
WT, n=5
Het, n=5
WT, n=11
Het, n=8
Blood glucose (mg/dL)
Time post-glucose injection (min)

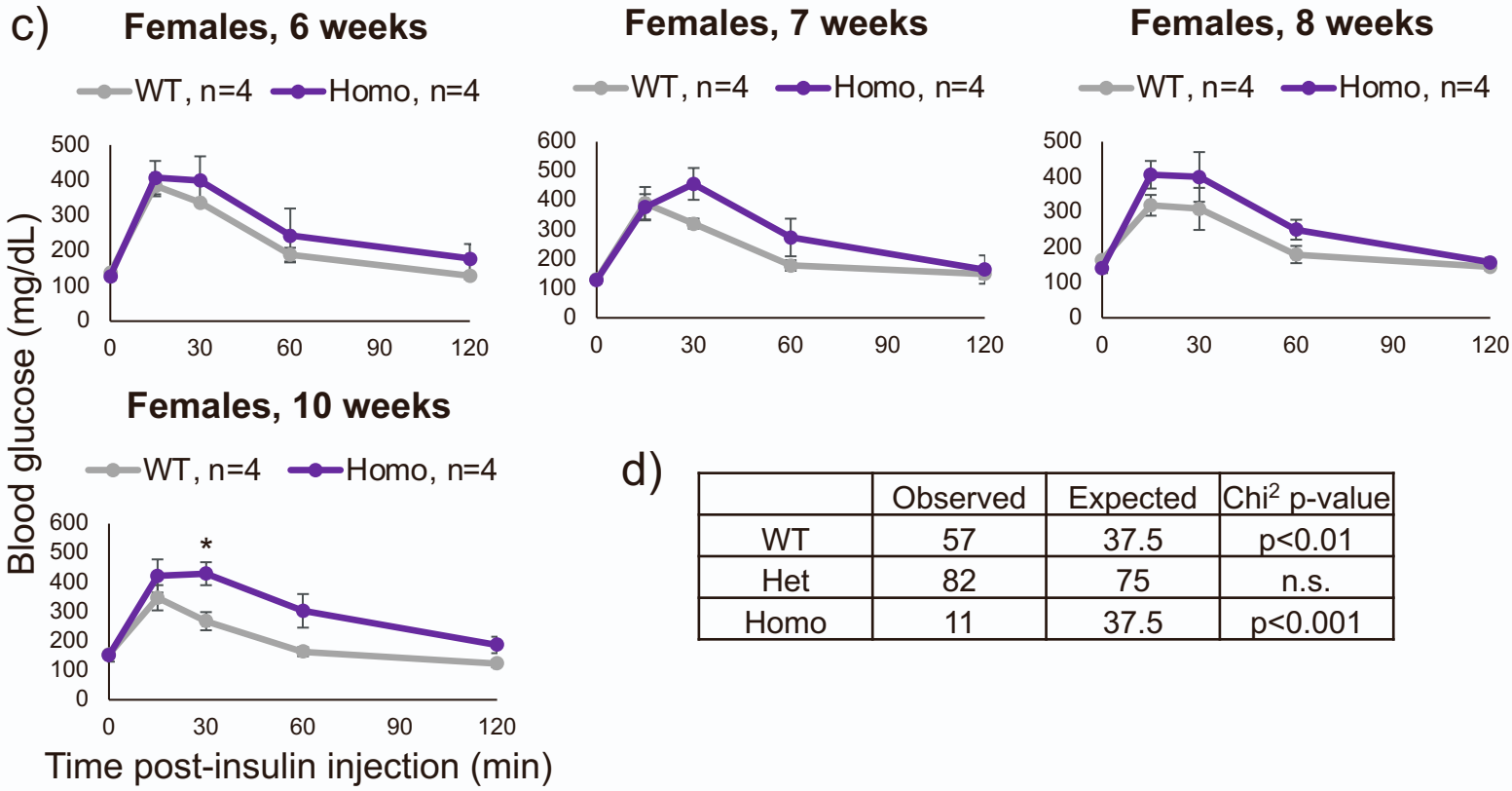
Figure S1: Glucose tolerance phenotypes were stably maintained in male and female *MafA*$^{S64F/+}$ mice. Related to Figure 1. GTT was performed in 3-, 6-, 7-, and 16-week-old mice. Glucose (2mg/kg) was injected following a 6 hour fast and blood glucose was measured at the indicated time points. Two-tail Student *t* test; *p<0.05; **p<0.01; ***p<0.001.

a)



b)

c)



d)

	Observed	Expected	Chi^2 p-value
WT	57	37.5	p<0.01
Het	82	75	n.s.
Homo	11	37.5	p<0.001

Figure S2: Homozygous male *MafA*$^{S64F/S64F}$ mice were hyperglycemic, with glucose tolerance and blood glucose levels worsening with age. Related to Figure 1. a) *MafA*$^{S64F/S64F}$ mice showed progressively worsening glucose tolerance and fasting blood glucose levels between 5 to 10 weeks of age. b) Fasting blood glucose levels in homozygous male mutant mice increased significantly over time. c) Female S64F MafA homozygous mice were only mildly glucose intolerant at 10 weeks of age although their temporal responses to glucose was variable between animals. d) Chi-square analysis revealed that *MafA*$^{S64F/S64F}$ male and female animals were observed with significantly less frequency at weaning than WT animals. In contrast, *MafA*$^{S64F/+}$ numbers were as expected. Two-tail Student *t* test; *p<0.05; **p<0.01; ***p<0.001.

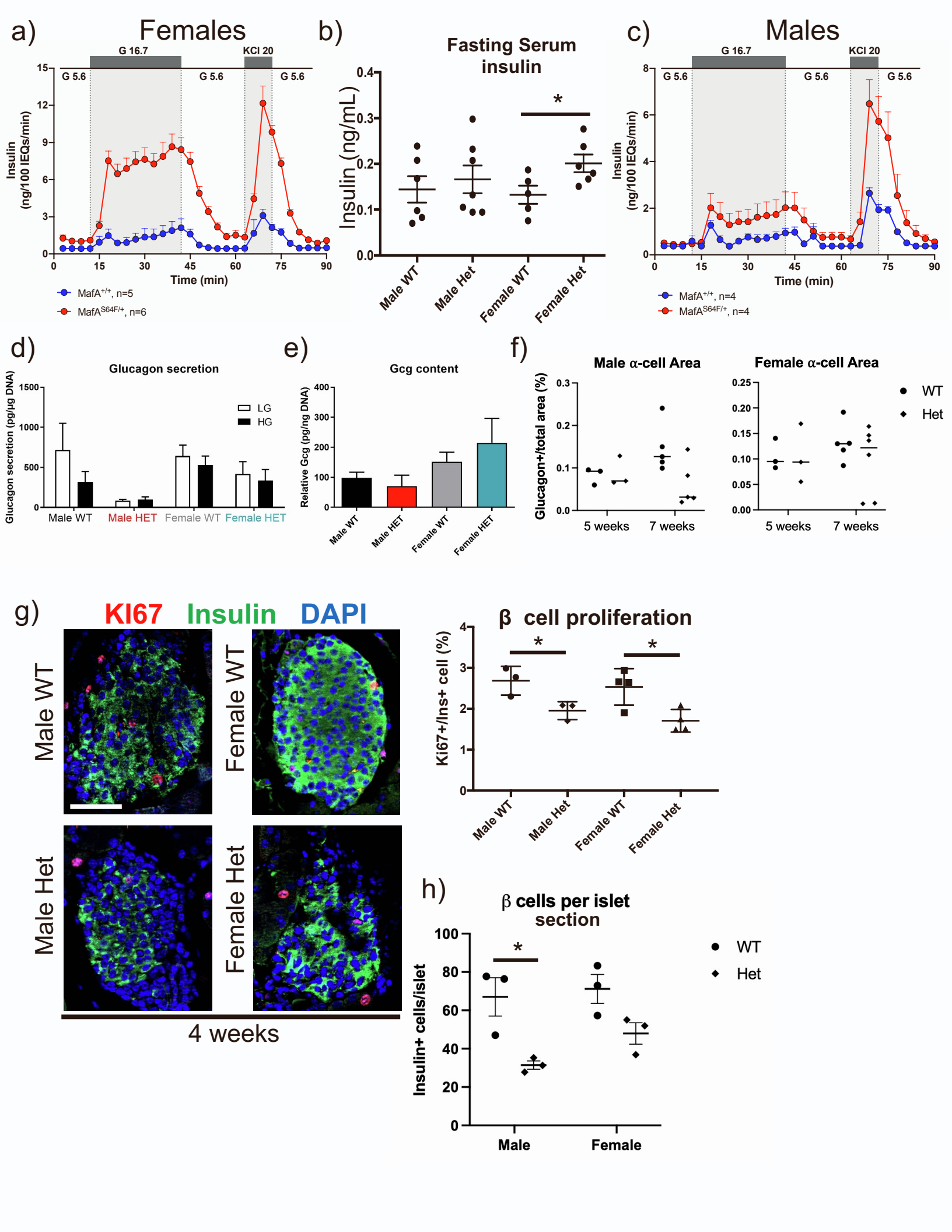

a)
Females
G 16.7
KCl 20
G 5.6
G 5.6
G 5.6
Insulin (ng/100 IEQs/min)
Time (min)
MafA+/+, n=5
MafAS64F/+, n=6
b)
Fasting Serum insulin
Insulin (ng/mL)
*
Male WT
Male Het
Female WT
Female Het
c)
Males
G 16.7
KCl 20
G 5.6
G 5.6
G 5.6
Insulin (ng/100 IEQs/min)
Time (min)
MafA+/+, n=4
MafAS64F/+, n=4
d)
Glucagon secretion
Glucagon secretion (pg/µg DNA)
LG
HG
Male WT
Male HET
Female WT
Female HET
e)
Gcg content
Relative Gcg (pg/ng DNA)
Male WT
Male HET
Female WT
Female HET
f)
Male α-cell Area
Female α-cell Area
Glucagon+/total area (%)
5 weeks
7 weeks
WT
Het
g)
KI67
Insulin
DAPI
Male WT
Female WT
Male Het
Female Het
4 weeks
β cell proliferation
Ki67+/Ins+ cell (%)
*
*
Male WT
Male Het
Female WT
Female Het
h)
β cells per islet section
Insulin+ cells/islet
*
Male
Female
WT
Het

Figure S3: Female and male *MafA*$^{S64F/+}$ islets secreted more insulin in response to high glucose with decreased proliferation and no change in glucagon secretion. Related to Figure 1. a) Islet perifusion demonstrated islets from *MafA*$^{S64F/+}$ female mice secreted higher levels of insulin at low (G 5.6) and high (G 16.7) glucose and in response to KCl. b) Serum insulin levels (ng/mL) were higher in 6-hour fasted female *MafA*$^{S64F/+}$ animals while male *MafA*$^{S64F/+}$ levels were unchanged. c) Islet perifusion results from *MafA*$^{S64F/+}$ male mice at high (G 16.7) glucose and in response to KCl. Islets were isolated from 8-10 week-old animals in both genders. Glucagon secretion (d) and content (e) were unchanged in *MafA*$^{S64F/+}$ islets, which were incubated in 4.6 mM (LG) and 16.7 mM (HG) glucose for 1 hour prior to collection. Secretion and content were normalized to DNA. f) Islet α cell area was not reduced in male or female *MafA*$^{S64F/+}$ mice, though measurements were highly variable between samples. Islet α cell area was calculated by dividing the total glucagon^{+} area by the total pancreas area (eosin staining) multiplied by 100 to obtain percent (%). Two-way ANOVA. g) Representative islets stained for insulin, Ki67 (proliferation marker), and DAPI (nuclei). Islet β cell proliferation was calculated by dividing the number of Ki67^{+} cells by total insulin^{+} β cells. Greater than 1000 β cells were counted per animal. h) β cell number per islet section was significantly reduced in *MafA*$^{S64F/+}$ male mice calculated by dividing the total number of Insulin+ cells by the number of islet sections (n ≥ 20 islets per sample) quantitated. Two-tail Student *t* test; *p<0.05.

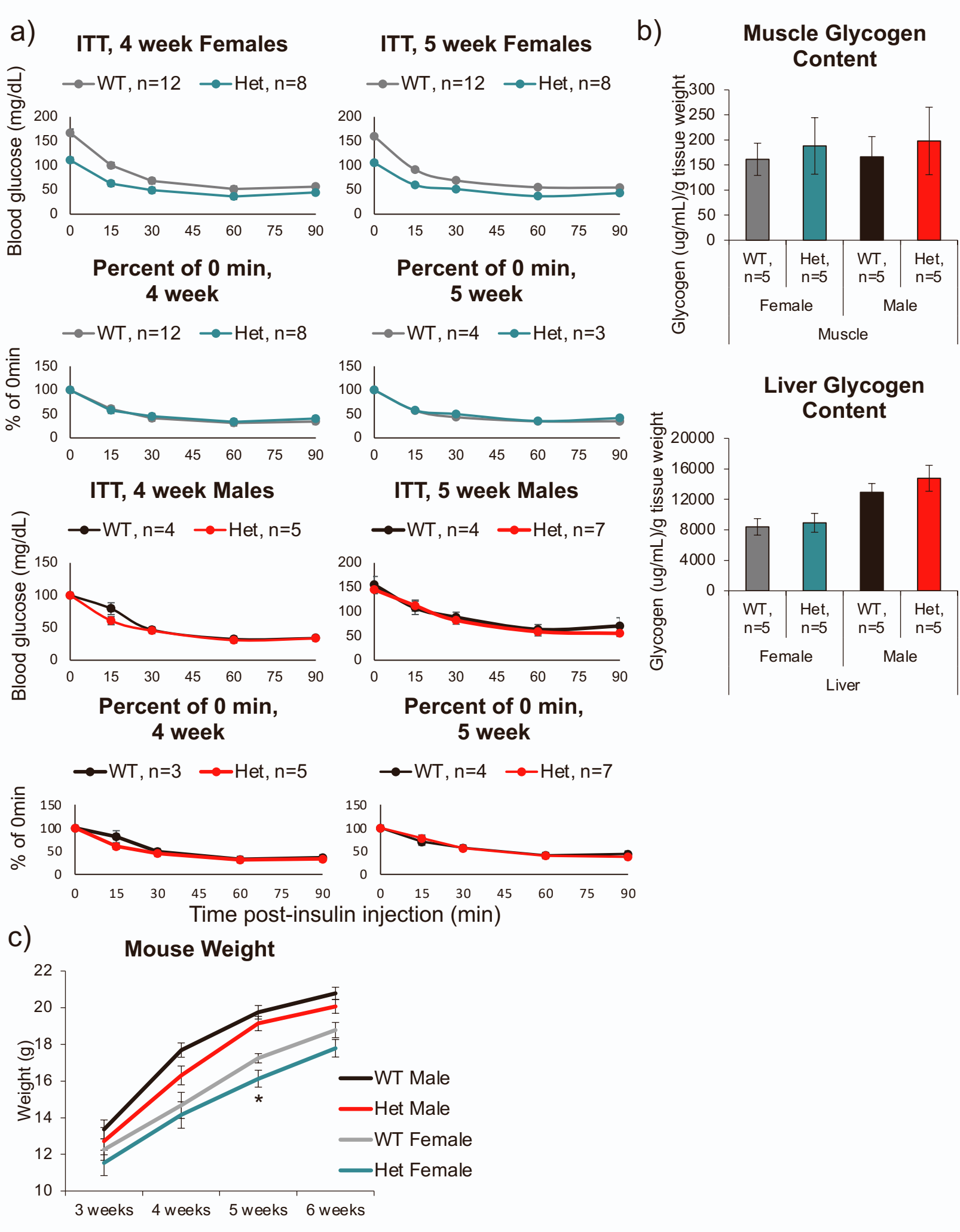
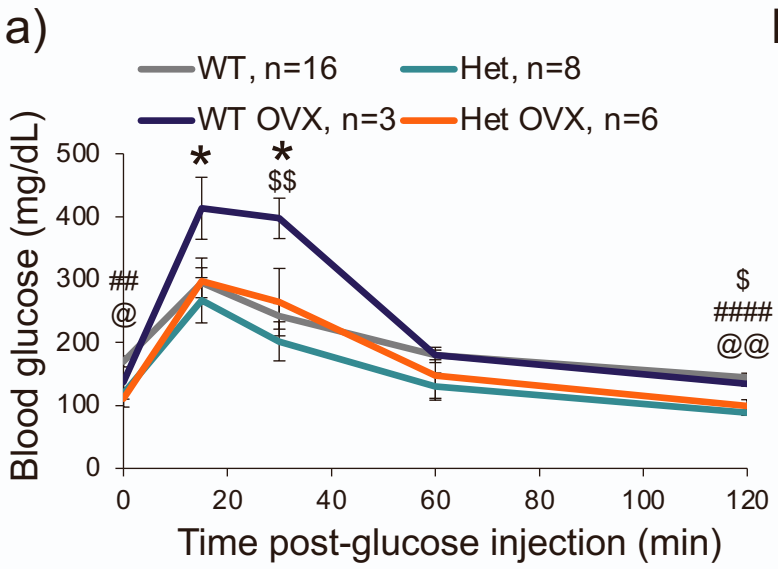
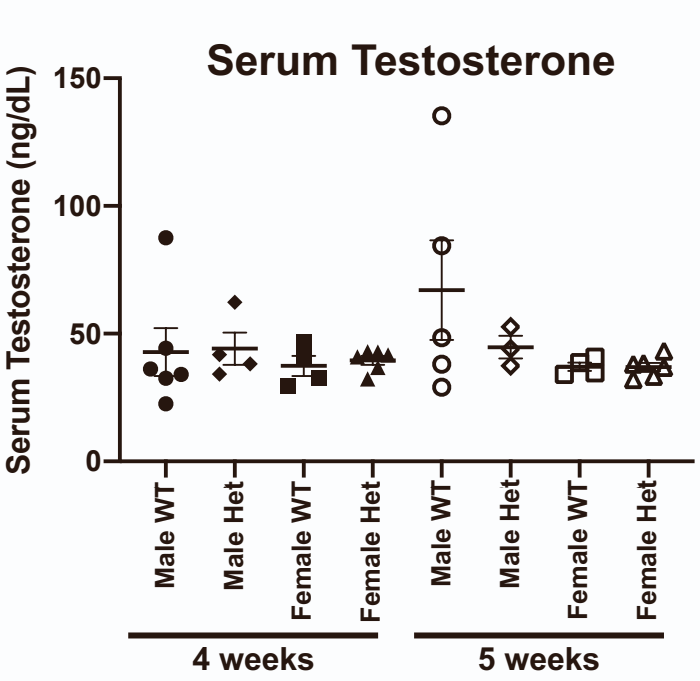
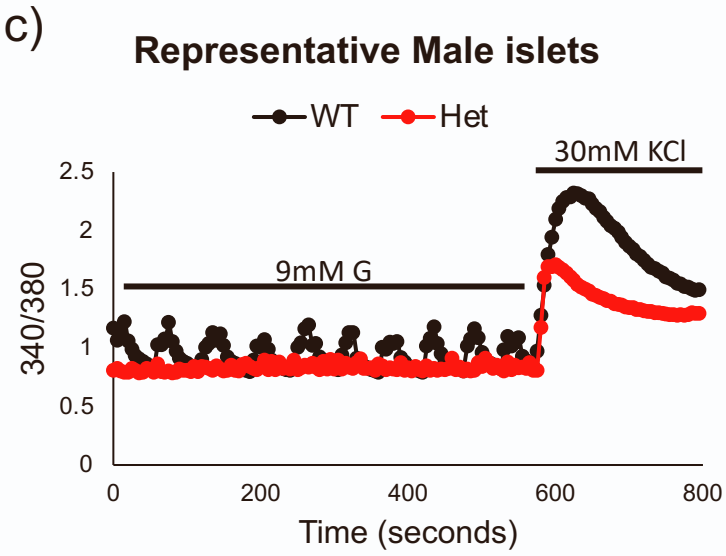
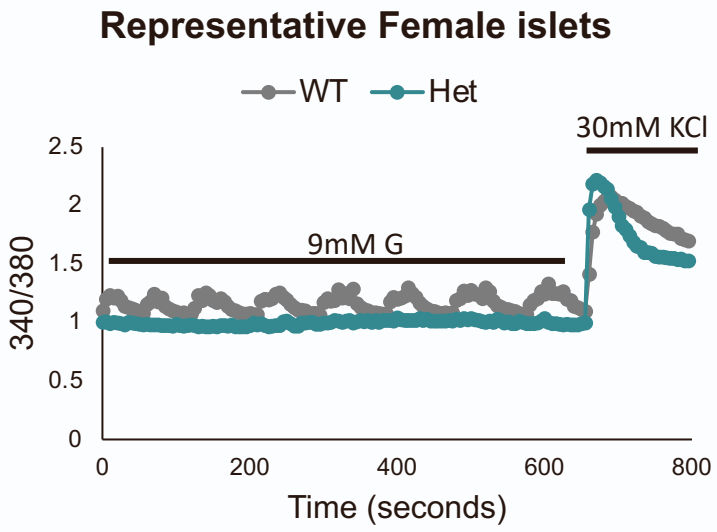

a)
ITT, 4 week Females
ITT, 5 week Females
WT, n=12
Het, n=8
Blood glucose (mg/dL)
Percent of 0 min, 4 week
Percent of 0 min, 5 week
WT, n=4
Het, n=3
% of 0min
ITT, 4 week Males
ITT, 5 week Males
WT, n=4
Het, n=5
Het, n=7
WT, n=3
Time post-insulin injection (min)
b)
Muscle Glycogen Content
Glycogen (ug/mL)/g tissue weight
WT, n=5
Het, n=5
Female
Male
Muscle
Liver Glycogen Content
Liver
c)
Mouse Weight
Weight (g)
3 weeks
4 weeks
5 weeks
6 weeks
WT Male
Het Male
WT Female
Het Female
*

Figure S4: Insulin tolerance and glycogen content are unaltered in *MafA*$^{S64F/+}$ animals. Related to Figure 1. a) Insulin tolerance was unchanged at 4 and 5 weeks in *MafA*$^{S64F/+}$ animals once corrected for initial fasted blood glucose levels (Percent of 0 min). Insulin (0.5U/kg body weight) was injected following a 6-hour fast. b) Glycogen content in muscle (soleus) and liver, normalized to wet tissue weight, was also unaffected in 7-week-old *MafA*$^{S64F/+}$ mice. c) There was no change in average body weight between 3-6 weeks except a small decrease (-1.07-fold) in female *MafA*$^{S64F/+}$ mice at 5 weeks of age. Two-tail Student *t* test; *p<0.05.


a)
WT, n=16
Het, n=8
WT OVX, n=3
Het OVX, n=6
Blood glucose (mg/dL)
Time post-glucose injection (min)
*
*
$$
##
@
$
####
@@


b)
Serum Testosterone
Serum Testosterone (ng/dL)
Male WT
Male Het
Female WT
Female Het
Male WT
Male Het
Female WT
Female Het
4 weeks
5 weeks


c)
Representative Male islets
WT
Het
30mM KCl
9mM G
340/380
Time (seconds)


Representative Female islets
WT
Het
30mM KCl
9mM G
340/380
Time (seconds)

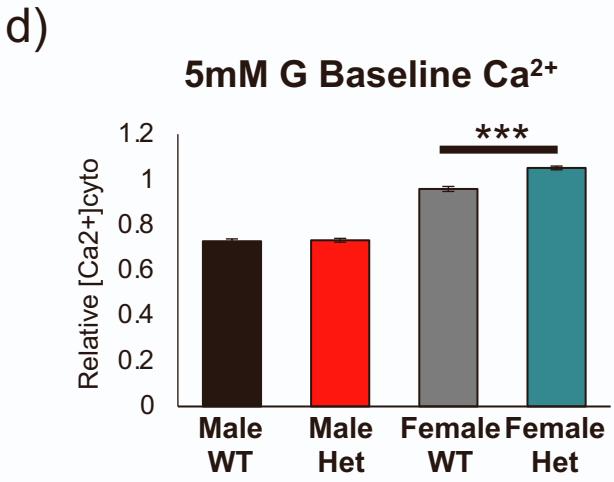

d)
5mM G Baseline Ca2+
***
Relative [Ca2+]cyto
Male WT
Male Het
Female WT
Female Het

Figure S5: Ovariectomy (OVX) did not alter glucose tolerance in female *MafA*$^{S64F/+}$ mice, nor are testosterone levels changed while male *MafA*$^{S64F/+}$ islets had a reduced response to KCl stimulation. Related to Figures 1 and 4. a) Compared to 4-week-old control mice (i.e., non OVX: WT, grey line; Het, teal line), the GTT at 1-week post ovariectomy showed glucose intolerance in WT female mice (WT OVX, purple line) and no change in *MafA*$^{S64F/+}$ mice (Het OVX, orange line). One-way ANOVA; WT vs. WT OVX: *p<0.05; Het vs. WT OVX: $^{\$}$p<0.05; $^{\$\$}$p<0.01; WT vs. Het: $^{\#\#}$p<0.01; $^{\#\#\#\#}$p<0.0001; WT vs. Het OVX: $^{@}$p<0.05; $^{@@}$p<0.01. b) Serum testosterone levels were unchanged in male or female *MafA*$^{S64F/+}$ mice. Male and female testosterone levels are similar at 4 and 5 weeks of age. Two-way ANOVA. c) Representative Ca^{2+} traces quantitated in Figure 4c in response to both high glucose (9 mM) and KCl (30 mM). d) Female *MafA*$^{S64F/+}$ islets have increased baseline Ca^{+2} at 5mM compared with WT islets (right) while male *MafA*$^{S64F/+}$ islets do not (left).

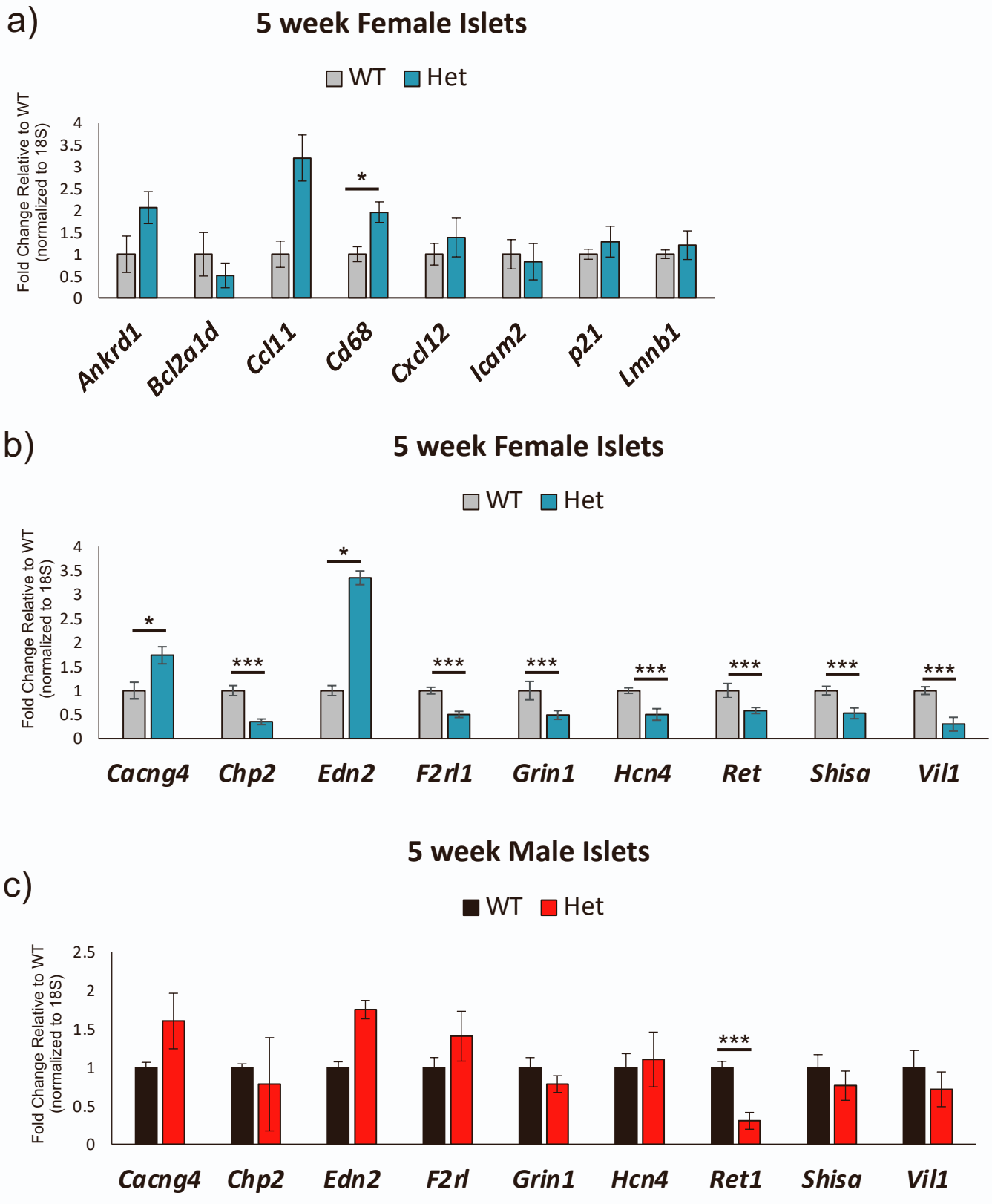


Figure S6: qPCR confirmation of changes in islet gene expression identified by RNA-seq. Related to Figure 5. a) Senescence markers were primarily unchanged in female *MafA*$^{S64F/+}$ islets. b-c) Genes altered specifically in female *MafA*$^{S64F/+}$ islets (b) identified by RNA-seq were mostly unchanged in male *MafA*$^{S64F/+}$ islets (c). Two-tail Student *t* test; *p<0.05; ***p<0.001.

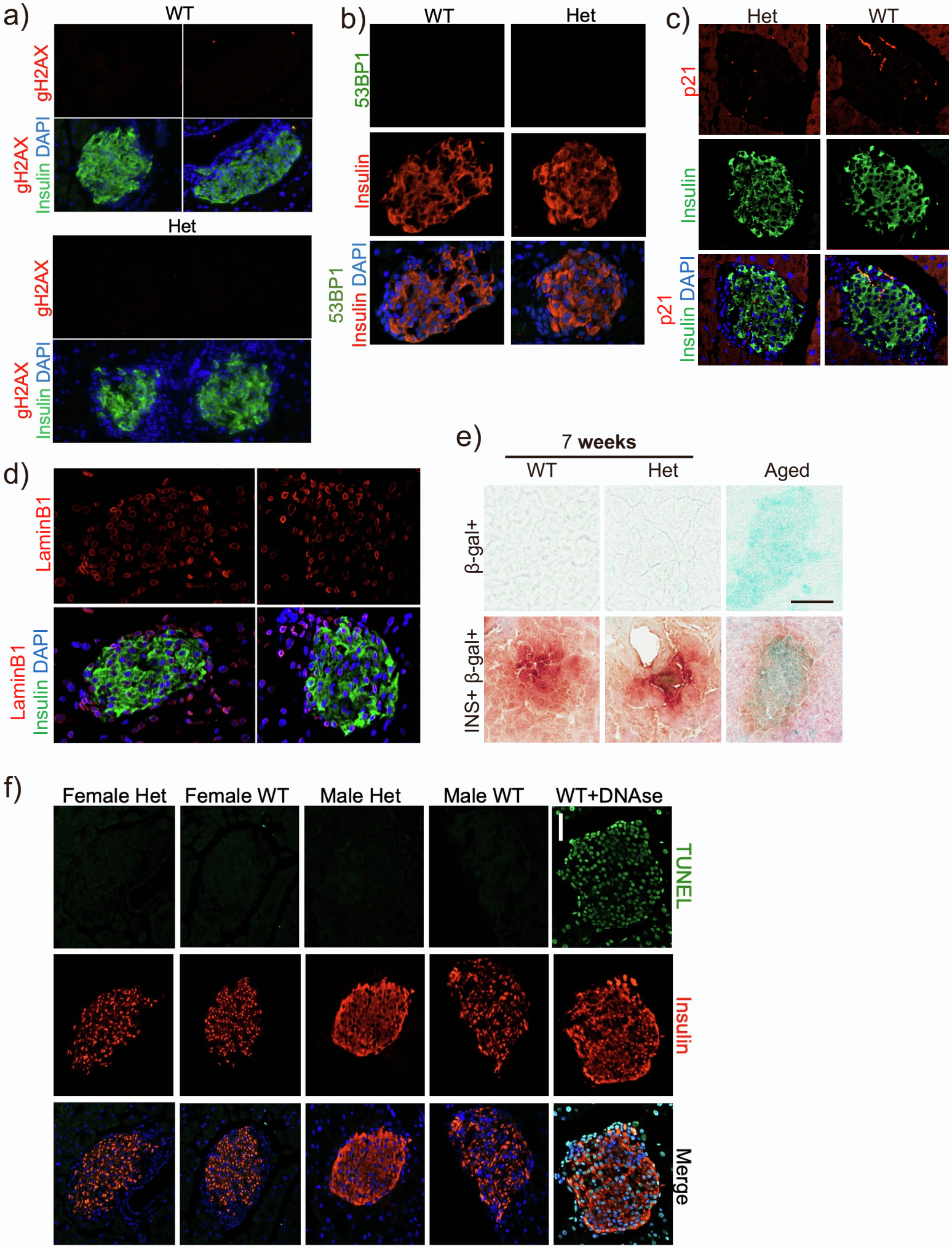

a)
WT
gH2AX
gH2AX
Insulin DAPI
Het
gH2AX
gH2AX
Insulin DAPI
b)
WT
Het
53BP1
Insulin
53BP1
Insulin DAPI
c)
Het
WT
p21
Insulin
p21
Insulin DAPI
d)
LaminB1
LaminB1
Insulin DAPI
e)
7 weeks
WT
Het
Aged
β-gal+
INS+ β-gal+
f)
Female Het
Female WT
Male Het
Male WT
WT+DNAse
TUNEL
Insulin
Merge

Figure S7: DNA damage and cell cycle inhibition markers were not detected in 5-week-old female *MafA*$^{S64F/+}$ islets, nor were significant levels of apoptosis found in *MafA*$^{S64F/+}$ islet cells. Related to Figure 6. a-e) Changes in immunostaining for γH2AX (DNA double strand break; a), 53BP1 (DNA double strand break; b), p21 (cell cycle inhibitor; c), LaminB1 protein (nuclear integrity marker; d) and endogenous staining for SA-β-gal (senescence marker; e) were not altered in female *MafA*$^{S64F/+}$ islets. f) TUNEL^{+} nuclei were barely detected in *MafA*$^{S64F/+}$ islets at 6 weeks of age but easily visible in DNase treated controls (top panels).

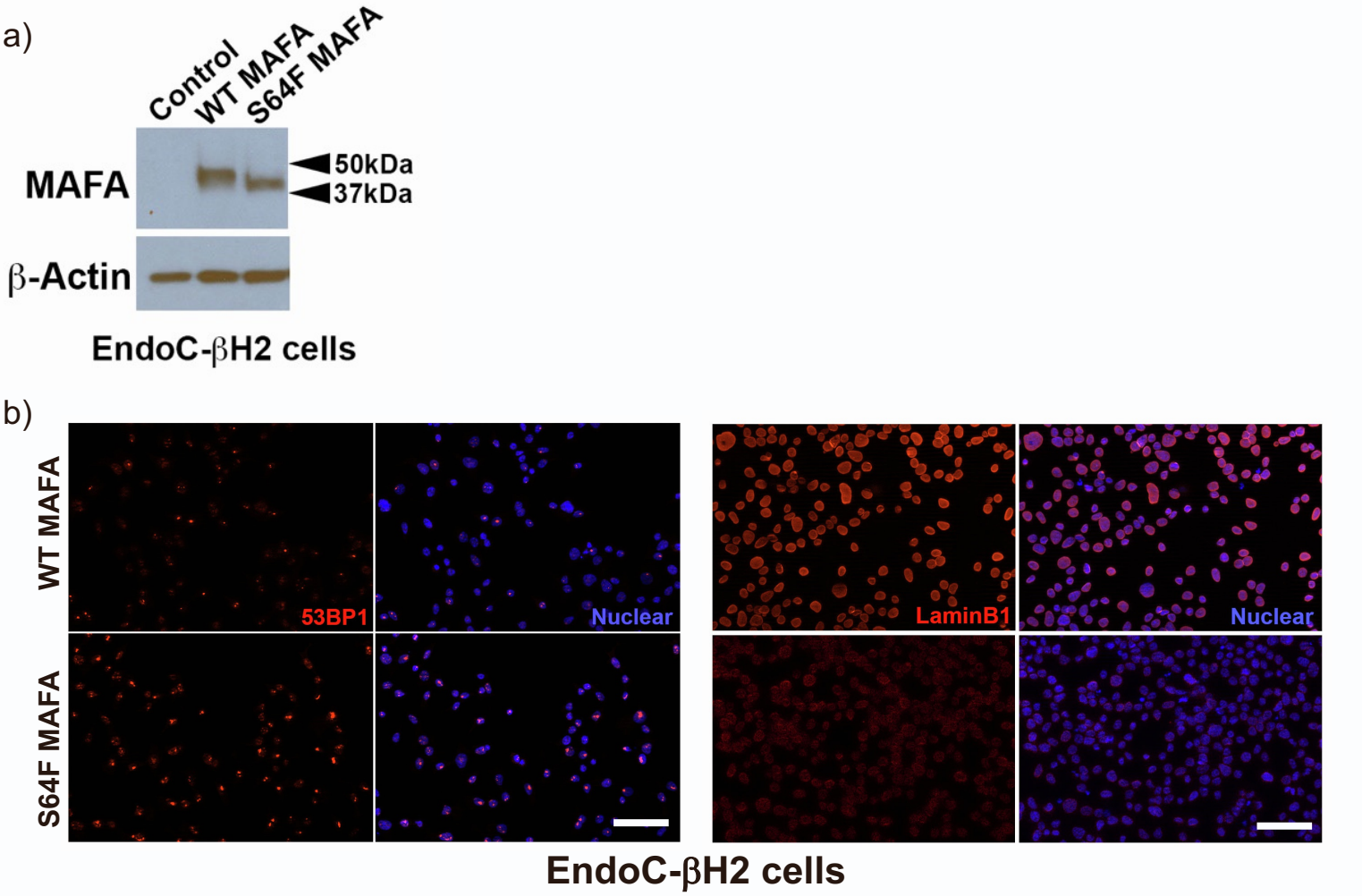


Figure S8: MAFA^{S64F} expressing human cells have changes in DNA damage, senescence, and Ca^{2+} channel gene expression. Related to Figure 7. a) Western blotting for EndoC-βH2 cells transduced to express WT MAFA or MAFA^{S64F} shows faster migration in the MAFA^{S64F} lane consistent with under-phosphorylated MAFA. Higher MAFA^{S64F} protein level is likely due to increased MAFA protein stability seen in (Iacovazzo et al., 2018). b) 53BP1 staining (left) intensity was higher and LaminB1 staining (right) intensity was lower in MAFA^{S64F} expressing EndoC-βH2 cells compared with MAFA^{WT}. c) qRT-PCR for Ca^{2+} channel genes (left) showed some alterations in MAFA^{S64F} expressing EndoC-βH2 cells while no changes in insulin secretion machinery genes were detected, consistent with findings in *MafA*$^{S64F/+}$ mouse islets.

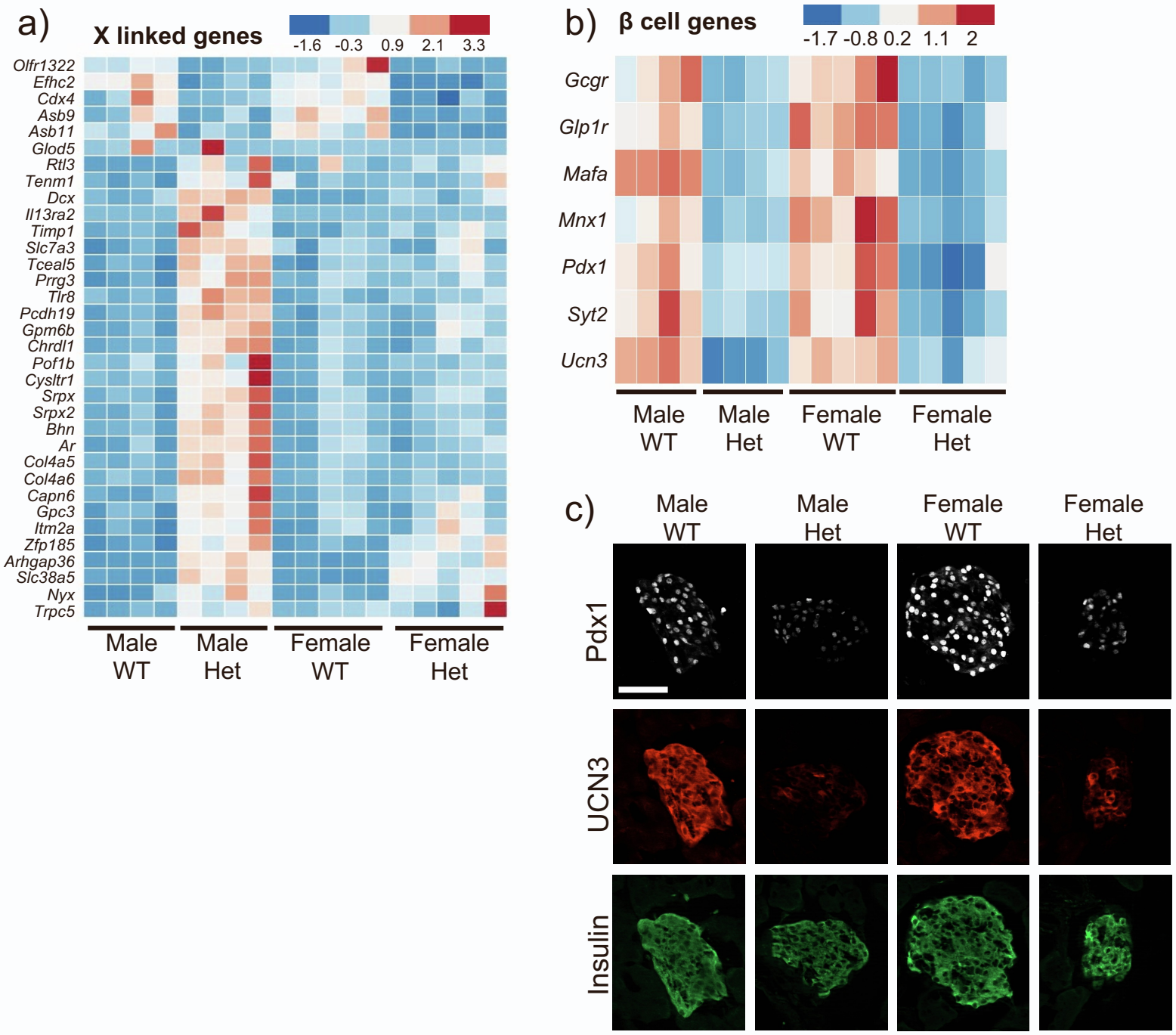


Figure S9: Many X chromosome linked genes were altered in male *MafA*$^{S64F/+}$ islets and production of some β cell identity gene products are downregulated in both male and female *MafA*$^{S64F/+}$ islets. Related to Figure 3. a) Heat map developed from the 5-week-old islet RNA-Seq data. FDR<0.05. b) Heat map showing β identity genes decreased in both male and female Het islets. FDR<0.05. c) Immunostaining illustrating decreased Pdx1 and Ucn3 protein levels in 7-week-old Het islets.

Table S1: Primer information

Mouse primers	Forward	Reverse
MafA	CCTGTAGAGGAAGCCGAGGAA	CCTCCCCCAGTCGAGTATAGC
Ins1	CACTTCCT ACCCCTGCTGG	ACCACAAAGATGCTGTTTGACA
pre-Ins2	GGGGAGCGTGGCTTCTTCTA	GGGGACAGAATTCAGTGGCA
Ins2	CCACCCAGGCTTTTGTCAAA	CCCAGCTCCAGTTGTTCCAC
Pdx1	CGGCTGAGCAAGCTAAGGTT	TGGAAGAAGCGCTCTCTTTGA
Ucn3	AGCACCCGGTACAGATACCAA	GGCCTTGTCGATGTTGAAGAG
Ankrd1	AAACGGACGGCACTCCACCG	CGCTGTGCTGAGAAGCTTGTCTCT
Bcl2a1d	GTATATCCACTCCCTGGCTGAG	TAGTCACAATCCTTCCCCAGTT
Ccl11	TCCACAGCGCTTCTATTCCTG	GGAGCCTGGGTGAGCCA
Cd68	ACTTCGGGCCATGTTTCTCT	GCTGGTAGGTTGATTGTCGT
Cxcl12	CAGTGACGGTAAACCAGTCAGC	TGGCGATGTGGCTCTCG
Icam1	CAATTTCTCATGCCGCACAG	AGCTGGAAGATCGAAAGTCCG
p21	GCCTTAGCCCTCACTCT TG	AGCTGGCCTTAGAGGTGACA
Cacng4	TCCGGAAGACGCGGACTAC	ATGATGTTGTGGCGTGTCTTG
Chp2	CGCCTAGACCTCCAGCAGATC	GCCTGCGAAATACAGTCTCTGAC
Edn2	CTGGCAAGATGTGGACTGCTGA	GCCTTTCTTGTCACCTCTGGCT
F2rl1	CGGACCGAGAACCTTGCACCG	GTGAGGATGGACGCAGAGAACT
Grin1	CCTTTCAGAGCACACTGTGGCT	CCAGGAAAACCACATGGCAGAG
Hcn4	CGTGCTCACTAAGGGCAACAAAG	GCACCTCATTGAAGTTGTCCACG
Ret	TTCCAGCATCAACTGCACTG	GTCAGTGGCTACCACCGTGT
Shisa2	TGCGACAACGACCGCCAGCAG	TGAAGGCAACGAACACTGAGCC
Vil1	TTCTACGGTGGTGACTGCTACC	TGGTCCAACAGGACGGCTTGAT
18S rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Lmnb1	CAGGAATTGGAGGACATGCT	GAAGGGCTTGGAGAGAGCTT
β-Actin	AGGTCATCACTATTGGCAACGA	CACTTCATGATGGAATTGAATGTAGTT

Human primers	Forward	Reverse
MAFA	GAGAGCGAGAAGTGCCAACT	TTCTCCTTGTACAGGTCCCG
MAFB	CATAGAGAACGTGGCAGCAA	ATGCCCGGAACTTTTTCTTT
INS	AGAGGCCATCAAGCAGATCACTGT	ACAGGTGTTGGTTCACAAAGGCTG
ANKRD1	AGACTCCTTCAGCCAACATGATG	CTCTCCATCTCTGAAATCCTCAGG
BCL2A1	GGATAAGGCAAAACGGAGGCTG	CAGTATTGCTTCAGGAGAGATAGC
CCL11	GCTACAGGAGAATCACCAGTGG	GGAATCCTGCACCCACTTCTTC
CD68	CGAGCATCATTCTTTCACCAGCT	ATGAGAGGCAGCAAGATGGACC
CXCL12	CTCAACACTCCAAACTGTGCCC	CTCCAGGTACTCCTGAATCCAC
ICAM1	AGCGGCTGACGTGTGCAGTAAT	TCTGAGACCTCTGGCTTCGTCA
P21	CCTGTCACTGTCTTGTACCCT	GCGTTTGGAGTGGTAGAAATCT
BCL2	ATCGCCCTGTGGATGACTGAGT	GCCAGGAGAAATCAAACAGAGGC
CXCL2	GGCAGAAAGCTTGTCTCAACCC	CTCCTTCAGGAACAGCCACCAA
ICAM3	AGATCGTCTGCAACGTGACCCT	TCGCTGAGGTTCACAATGGGTC
IGFBP2	CGAGGGCACTTGTGAGAAGCG	TGTTCATGGTGCTGTCCACGTG
IGFBP4	GAGCTGGGTGACACTGCTTG	CCCACGAGGACCTCTACATCA
IGF1R	CTCCTGTTTCTCTCCGCCG	ATAGTCGTTGCGGATGTCGAT
LMNB1	TGGAGTGG TTGTTGAGGAAG	GAGAAGGCTCTGCACTGTATAC
CACNA1A	CTGGTAGCCTTTGCCTTCACTG	ACACAGCCTTGAGCTTTGGCAG
CACNA1C	GCAGGAGTACAAGAACTGTGAGC	CGAAGTAGGTGGAGTTGACCAC
CACNA1E	AGCGTGAGACAGGCAAAGCCAT	GGATGCACATCTCAAAGTAGCGC
CACNA1G	TTCACCGCAGTCTTTCTGGCTG	TGACGGAGATGAGCACCAACAG
EXOC3	GAGCCATTGCTTTCTCCACACG	TGGCTCTGTCTCTTTGACCCAG
EXOC5	CTTCAGTAATCCAGAAACAGTCCT	TGCTCTGCATCGGACTTCCTAC
SNAP25	CGTCGTATGCTGCAACTGGTTG	GGTTCATGCCTTCTTCGACACG
VAMP2	CTCCAAACCTCACCAGTAACAGG	AGCTCCGACAGCTTCTGGTCTC
STX1A	GGAACACGCGGTAGACTATGT	CTGGAGTGGAGTGGCAGTTT
KCNJ11	TGTGTCACCAGCATCCACTCCT	GTTCTGCACGATGAGGATCAGG
GAPDH	CTCACCGGATGCACCAATGTT	CGCGTTGCTCACAATGTTCAT