Supplemental Information

Supplementary Figure 1. Adenovirus-mediated shCyb5r3 Knockdown, Related to Figure 2. (A) *Cyb5r3* in Min6 cells transduced with shCyb5r3 *vs*. shScramble at varying MOIs. (B) *Cyb5r4* mRNA in the same samples as (A). (C) Densitometry of Cyb5r3 protein levels in samples transduced with shScr vs. varying MOIs of shCyb5r3 adenovirus. (D) Western blot used for densitometry shown in (C). Rat Ins1 cells were used as a negative control for the mouse-specific shCyb5r3 sequence. (E) *Cyb5r3* mRNA in WT islets transduced with Ad-shScramble vs. Ad-shCyb5r3. All data are presented as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 by Student's t-test. All experiments were performed at least 3 times in triplicate.

Supplementary Figure 2. Metabolic Phenotyping of B-Cyb5r3 mice, Related to Figure 4. (A-B) Body composition measured by MRI of B-Cyb5r3 vs. RIP-Cre^{+} mice fed (A) normal chow and (B) HFD. (C-D) Oral glucose tolerance test (OGTT) of 4-month-old B-Cyb5r3 (C) female and (D) male mice fed normal chow. (E) Fasting and refed triglycerides and (F) non-esterified free fatty acids (NEFAs) in B-Cyb5r3 mice on normal chow. All data are presented as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 by Student's t-test (n=4-6 per group).

Supplementary Figure 3. Cyb5r3 Expression and IPGTT in Male B-Cyb5r3 Mice, Related to Figures 3 and 4. (A) Immunostaining of Insulin (red), Cyb5r3 (green), Pdx1 (blue), and DAPI (white) in male B-Cyb5r3 pancreata (n=3 per group). (B) IPGTT in 8-month-old male B-Cyb5r3 mice vs. RIP-Cre^{+} controls (n=4 per group). All data are presented as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 by Student's t-test.

Supplementary Figure 4. Islet Cell Quantification, Related to Figures 3 and 6. (A) Insulin, Glucagon, and Somatostatin + PP area normalized to total islet area as determined by immunohistochemistry. Data shown for 2-month-old and 6-month-old B-Cyb5r3 mice vs. RIP-Cre^{+} controls. All data are presented as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 by Student's t-test, (n=3 per group).

Supplementary Figure 5. Forest plot of disease associations with rs5758837.

The PheWAS graphic displays associations for a SNP in the *CYB5R3* promoter, rs5758837, across all phenotypes included in the Type 2 Diabetes Knowledge Portal, or across UK Biobank phenotypes [29].

Supplementary Table 1 List of Antibodies Used

Antigen	Species	Dilution	Product Number	Company
Insulin	Guinea Pig	1:2000	A0564	DAKO
Aldh1a3	Rabbit	1:100	NBP2-15339	Novus
Cyb5r3	Rabbit	1:100	10894-1-AP	Proteintech
Proglucagon	Rabbit	1:500	#8233	Cell Signaling
Somatostatin	Goat	1:1000	SC-7819	Santa Cruz
Pancreatic Polypeptide	Goat	1:500	AB77192	Abcam
Glut2	Rabbit	1:200	AB54460	Abcam
MafA	Rabbit	1:200	IHC00352	Bethyl
Pdx1	Goat	1:100	AF2419	R&D
FoxO1	Rabbit	1:100	CST 2880S	Cell Signaling
Neurogenin3	Rabbit	1:100	AB2011	BCBC

Figure S1

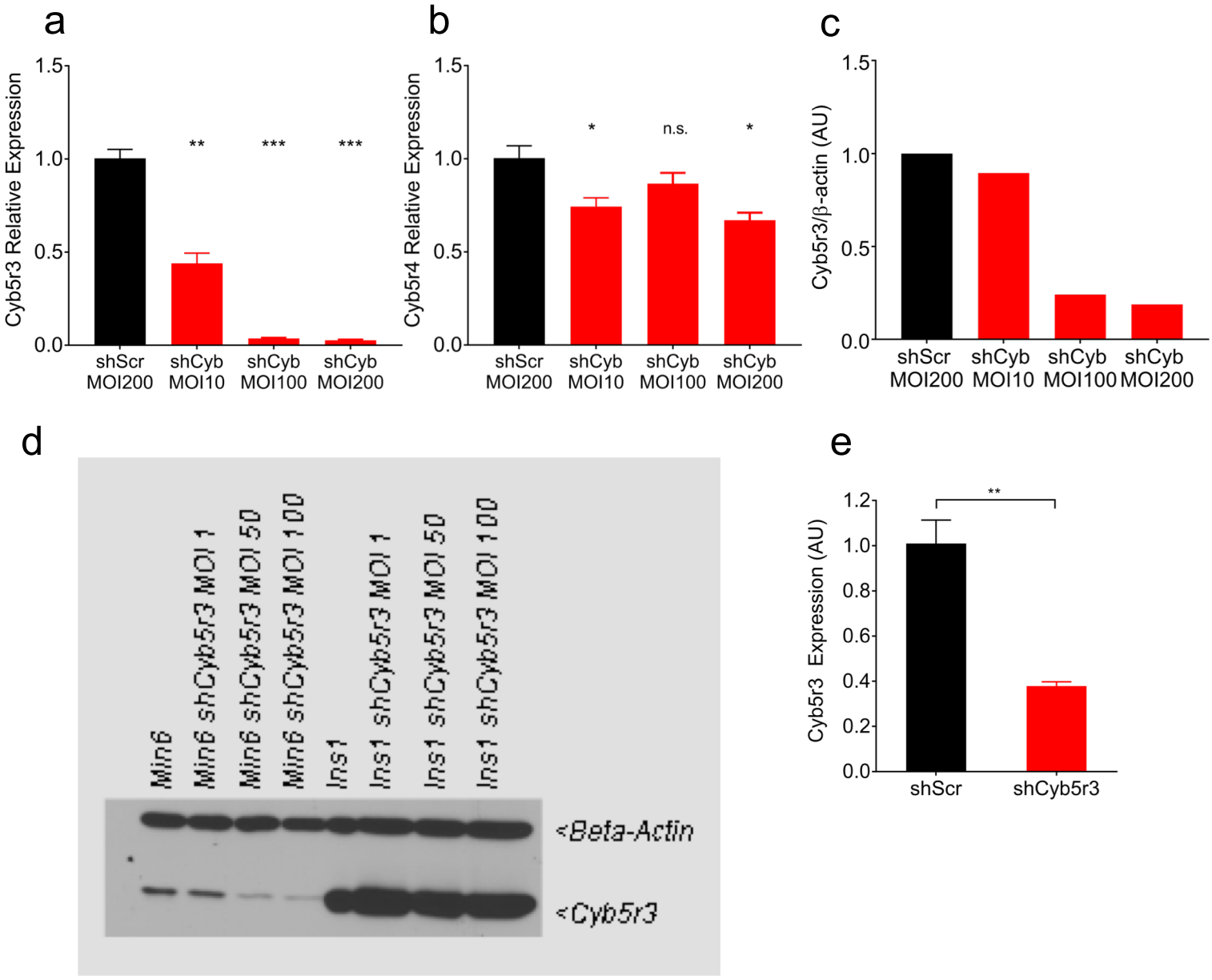

a
Cyb5r3 Relative Expression
1.5
1.0
0.5
0.0
**
***
***
shScr MOI200
shCyb MOI10
shCyb MOI100
shCyb MOI200
b
Cyb5r4 Relative Expression
1.5
1.0
0.5
0.0
*
n.s.
*
shScr MOI200
shCyb MOI10
shCyb MOI100
shCyb MOI200
c
Cyb5r3/β-actin (AU)
1.5
1.0
0.5
0.0
shScr MOI200
shCyb MOI10
shCyb MOI100
shCyb MOI200
d
Min6
Min6 shCyb5r3 MOI 1
Min6 shCyb5r3 MOI 50
Min6 shCyb5r3 MOI 100
Ins1
Ins1 shCyb5r3 MOI 1
Ins1 shCyb5r3 MOI 50
Ins1 shCyb5r3 MOI 100
<Beta-Actin
<Cyb5r3
e
Cyb5r3 Expression (AU)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
shScr
shCyb5r3

Figure S2

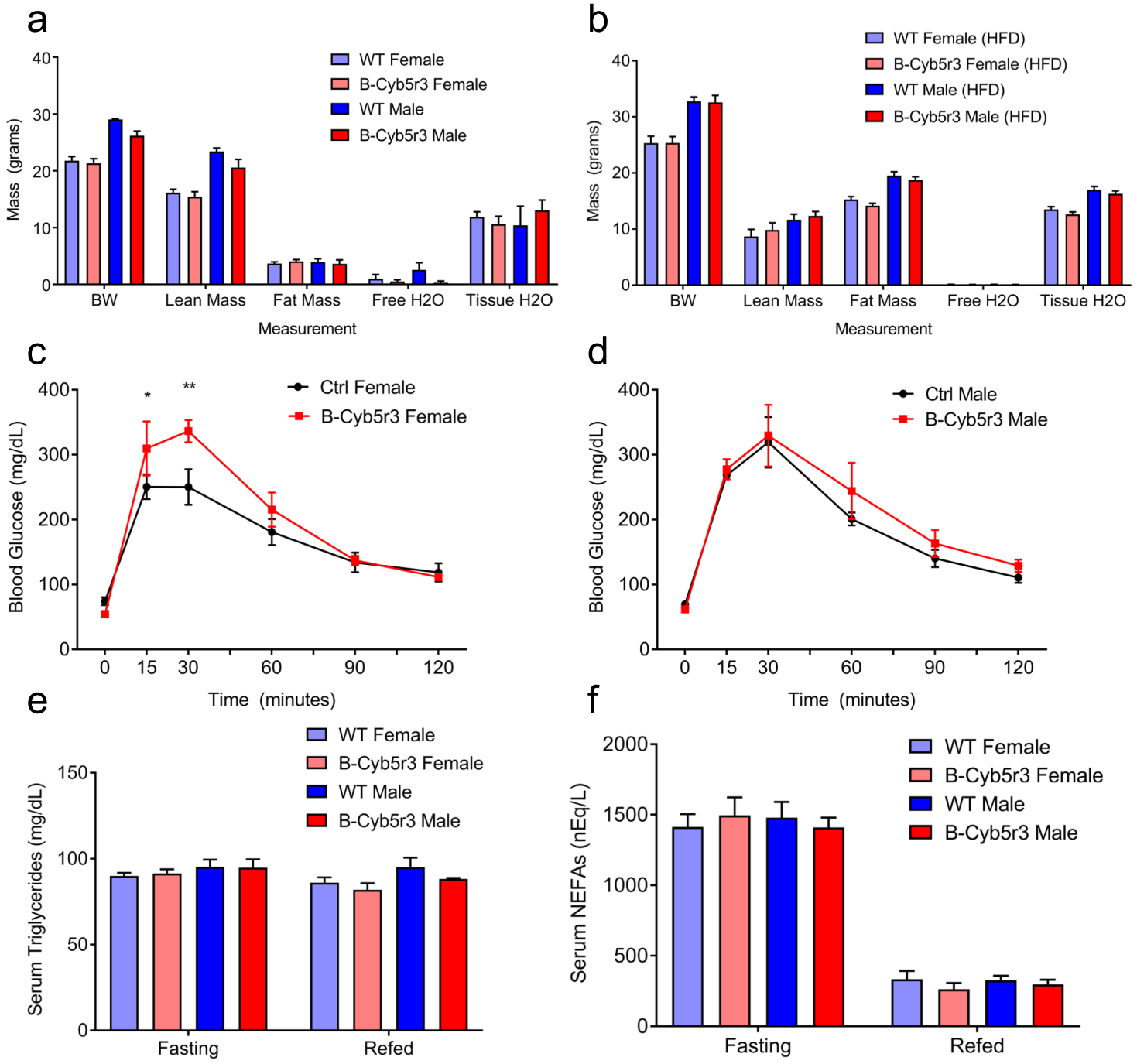
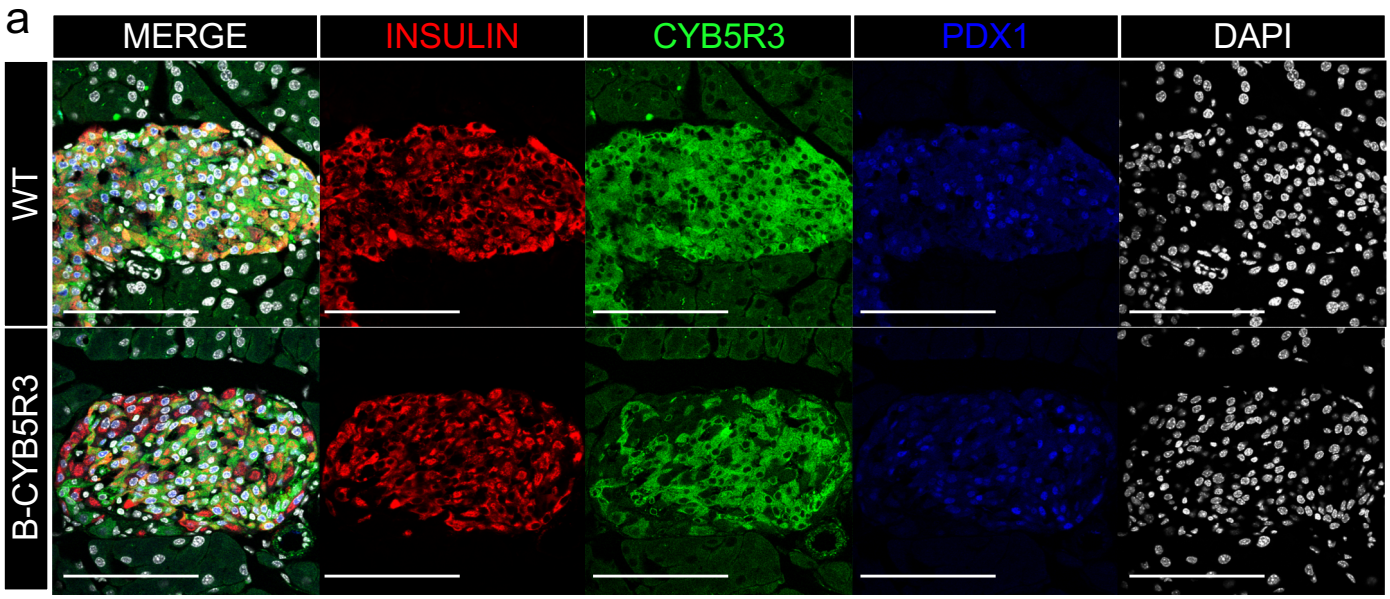

a
Mass (grams)
WT Female
B-Cyb5r3 Female
WT Male
B-Cyb5r3 Male
BW
Lean Mass
Fat Mass
Free H2O
Tissue H2O
Measurement
b
WT Female (HFD)
B-Cyb5r3 Female (HFD)
WT Male (HFD)
B-Cyb5r3 Male (HFD)
c
Blood Glucose (mg/dL)
Ctrl Female
B-Cyb5r3 Female
Time (minutes)
d
Ctrl Male
B-Cyb5r3 Male
e
Serum Triglycerides (mg/dL)
Fasting
Refed
f
Serum NEFAs (nEq/L)

Figure S3


a
MERGE
INSULIN
CYB5R3
PDX1
DAPI
WT
B-CYB5R3

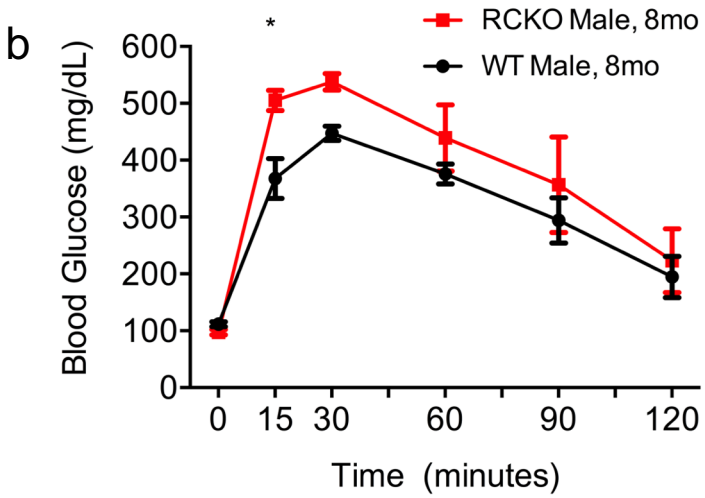

b
RCKO Male, 8mo
WT Male, 8mo
*
Blood Glucose (mg/dL)
600
500
400
300
200
100
0
0
15
30
60
90
120
Time (minutes)

Figure S4

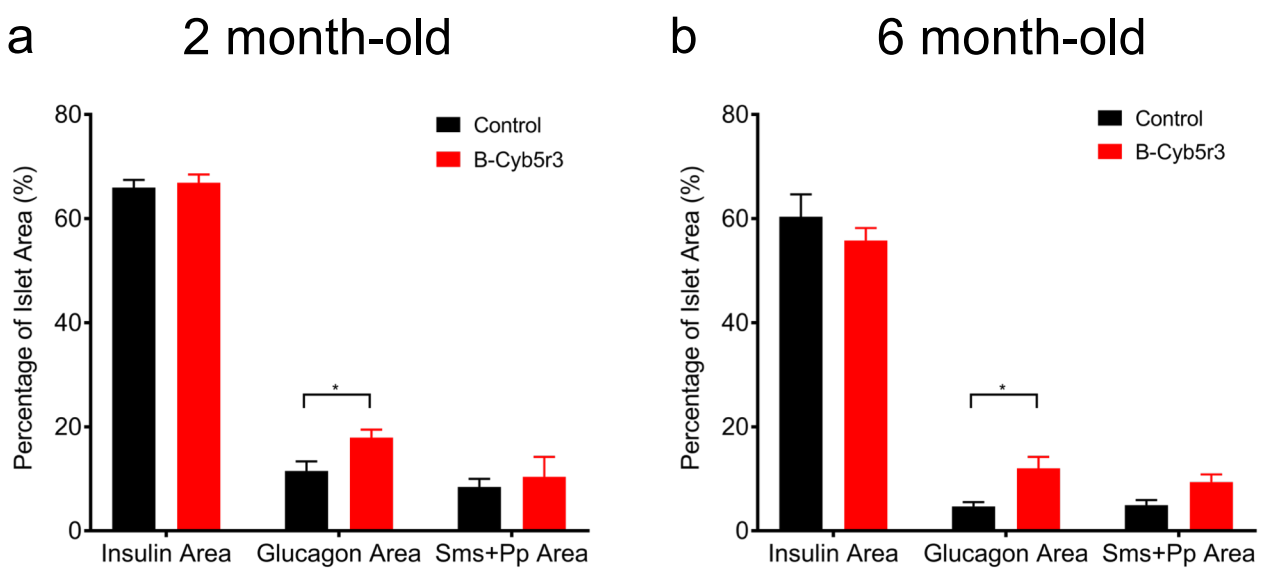
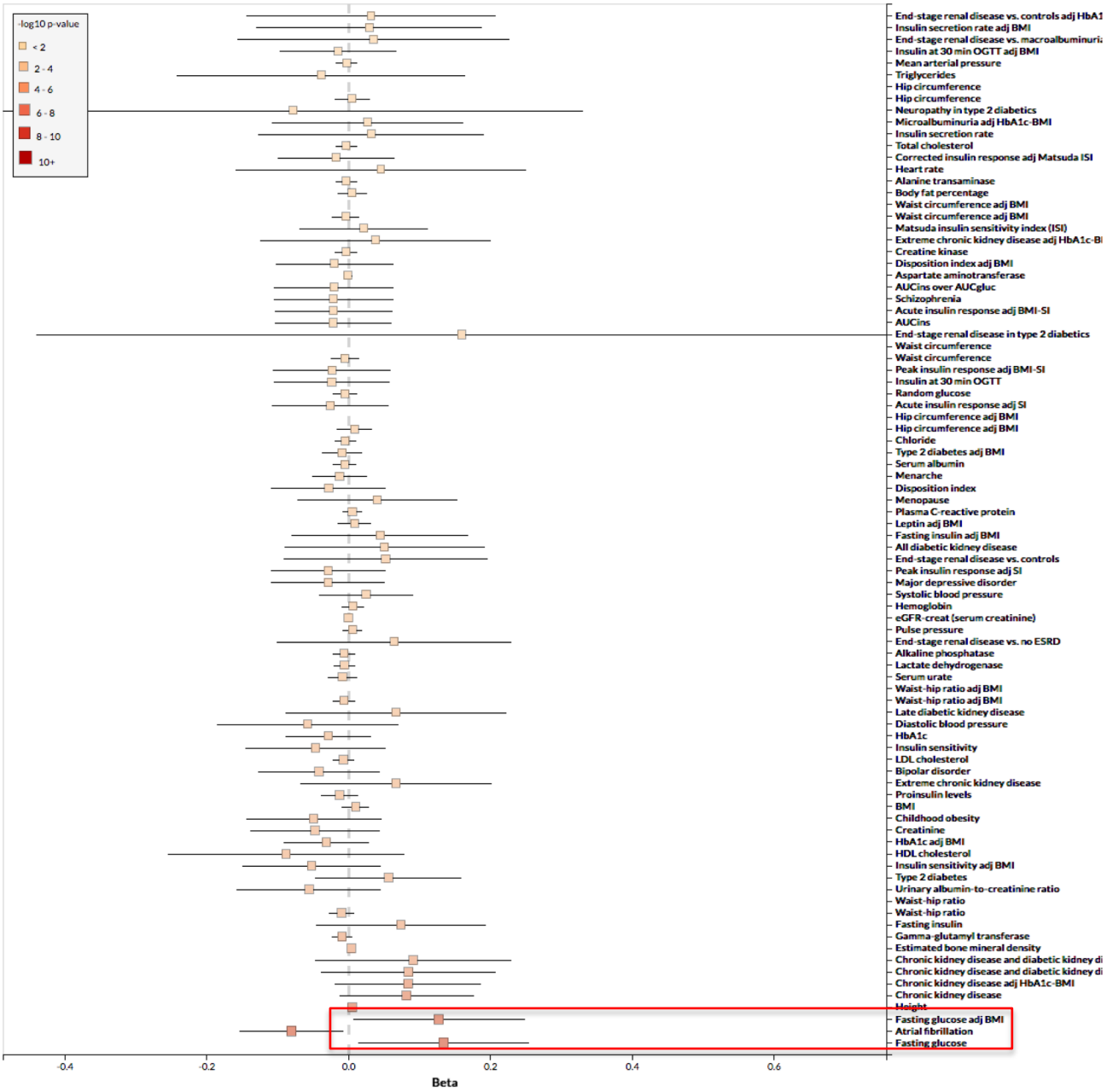

a
2 month-old
b
6 month-old
Control
B-Cyb5r3
Percentage of Islet Area (%)
80
60
40
20
0
*
Insulin Area
Glucagon Area
Sms+Pp Area

Figure S5


-log10 p-value
< 2
2 - 4
4 - 6
6 - 8
8 - 10
10+
End-stage renal disease vs. controls adj HbA1
Insulin secretion rate adj BMI
End-stage renal disease vs. macroalbuminuri
Insulin at 30 min OGTT adj BMI
Mean arterial pressure
Triglycerides
Hip circumference
Hip circumference
Neuropathy in type 2 diabetics
Microalbuminuria adj HbA1c-BMI
Insulin secretion rate
Total cholesterol
Corrected insulin response adj Matsuda ISI
Heart rate
Alanine transaminase
Body fat percentage
Waist circumference adj BMI
Waist circumference adj BMI
Matsuda insulin sensitivity index (ISI)
Extreme chronic kidney disease adj HbA1c-BI
Creatine kinase
Disposition index adj BMI
Aspartate aminotransferase
AUCins over AUCgluc
Schizophrenia
Acute insulin response adj BMI-SI
AUCins
End-stage renal disease in type 2 diabetics
Waist circumference
Waist circumference
Peak insulin response adj BMI-SI
Insulin at 30 min OGTT
Random glucose
Acute insulin response adj SI
Hip circumference adj BMI
Hip circumference adj BMI
Chloride
Type 2 diabetes adj BMI
Serum albumin
Menarche
Disposition index
Menopause
Plasma C-reactive protein
Leptin adj BMI
Fasting insulin adj BMI
All diabetic kidney disease
End-stage renal disease vs. controls
Peak insulin response adj SI
Major depressive disorder
Systolic blood pressure
Hemoglobin
eGFR-creat (serum creatinine)
Pulse pressure
End-stage renal disease vs. no ESRD
Alkaline phosphatase
Lactate dehydrogenase
Serum urate
Waist-hip ratio adj BMI
Waist-hip ratio adj BMI
Late diabetic kidney disease
Diastolic blood pressure
HbA1c
Insulin sensitivity
LDL cholesterol
Bipolar disorder
Extreme chronic kidney disease
Proinsulin levels
BMI
Childhood obesity
Creatinine
HbA1c adj BMI
HDL cholesterol
Insulin sensitivity adj BMI
Type 2 diabetes
Urinary albumin-to-creatinine ratio
Waist-hip ratio
Waist-hip ratio
Fasting insulin
Gamma-glutamyl transferase
Estimated bone mineral density
Chronic kidney disease and diabetic kidney di
Chronic kidney disease and diabetic kidney di
Chronic kidney disease adj HbA1c-BMI
Chronic kidney disease
Height
Fasting glucose adj BMI
Atrial fibrillation
Fasting glucose
-0.4
-0.2
0.0
0.2
0.4
0.6
Beta