Appendix A: Supplemental Figures for Chapter 2



Supplemental Figure 1. *Atp10A* KO female mice have smaller bodies on the 12th week of the HFD. (A) Body length of mice was measured after CO₂ sacrifice. P value by unpaired t-test, **P=0.0094. (Male: 10A^{+/+} n=11, 10A^{-/-} n=5; Female: 10A^{+/+} n=17, 10A^{-/-} n=14). (B)The wet mass of tissue was measured after removal from female mice on the 12th week on the HFD. Tissue mass is normalized to body mass. P value by unpaired t-tests. (10A^{+/+} n=3-5,10A^{-/-} n=7).

	<u>10A+/+ (WT) Average (n=7)</u>			<u>10A-/- (KO) Average (n=13)</u>			P-value (t-test, WT vs KO)		
Females	<u>Light</u>	<u>Dark</u>	<u>Full day</u>	Light	<u>Dark</u>	<u>Full day</u>	Light	<u>Dark</u>	Full day
Avg. Daily Food intake (kcal/period)	6.5	9.5	14.4	5.4	9.5	13.5	0.0353 (*)	0.9515 (ns)	0.4425 (ns)
Avg. Daily Energy Expenditure (kcal/period)	17.3	28.9	46.1	17.4	28.4	45.7	0.9596 (ns)	0.5622 (ns)	0.7033 (ns)
<u>Total Food (kcal)</u>	47.3	57.5	57.5	43.7	54.1	54.1	0.2807 (ns)	0.4428 (ns)	0.4428 (ns)
O2 Consumption (ml/hr)	102.9	125	115.5	103.3	123.1	114.6	0.8895 (ns)	0.5891 (ns)	0.7477 (ns)
CO2 Production (ml/hr)	81	98.1	90.8	80.3	95.9	89.2	0.7319 (ns)	0.4586 (ns)	0.5217 (ns)
Energy balance (kcal)	2.1	9.3	11.5	-1.2	9.6	8.5	0.0345 (*)	0.9394 (ns)	0.4886 (ns)
Resipiratory Exchange Ratio	0.8	0.8	0.8	0.8	0.8	0.8	0.0839 (ns)	0.3642 (ns)	0.1761 (ns)
Locomotor Activity (beam breaks/hr)	121.5	269.9	206.3	100.9	266.1	195.3	0.2581 (ns)	0.8697 (ns)	0.5679 (ns)
Pedestrian Locomotion (m)	146.9	880.8	1027.7	141.1	843	984	0.8056 (ns)	0.7245 (ns)	0.7222 (ns)
Distances in cage Locomotion (m)	199.6	1017.4	1217	185.5	980.6	1166	0.5972 (ns)	0.7443 (ns	0.6956 (ns)

Supplemental Figure 2. *Atp10A* deletion causes alterations to average daily food intake and energy balance during the light hours in female mice fed a HFD for 13 weeks. The table indicates the parameters measured in the Sable System's Promethion Indirect Calorimetry System where the mice were single-housed. The values are an average measurement from 5 days in the system with ad libitum feeding. P value by unpaired t-test ($10A^{+/+}$ n=7, $10A^{-/-}$ n=13).



Supplemental Figure 3. *Atp10A* deletion does not affect weight gain, body composition, or glucose homeostasis in female mice after 12 weeks on normal chow. (A) Weight gain of $10A^{+/+}$ and $10A^{-/-}$ female mice over the course of 12 weeks on normal chow (4.5 kcal% fat, Ad lib feeding), P value by 2-way ANOVA with Sidak's multiple comparison. ($10A^{+/+}$ n=4, $10A^{-/-}$ n=7). (B) Lean and (C) fat body mass was normalized to total body mass to calculate % Lean and % Fat mass. P value by unpaired t-test ($10A^{+/+}$ n=4, $10A^{-/-}$ n=4). (D) Body length of mice was measured after CO₂ sacrifice. P value by unpaired t-test ($10A^{+/+}$ n=4, $10A^{-/-}$ n=4). (E) Fasting blood glucose was measured a 5 hour fast, via a glucometer. P value by unpaired t-test ($10A^{+/+}$ n=4, $10A^{-/-}$ n=4). (F) Fasting plasma insulin was measured aftr a 5 hour fast. P-value by unpaired t test ($10A^{+/+}$ n=4, $10A^{-/-}$ n=4).



Supplemental Figure 4. Maternally inherited *Atp10A* deletion leads to smaller bodies and elevated fasting blood glucose in male mice on the 12th week on the HFD. (A) Weight gain of heterozygous males and females inheriting the KO allele maternally (Maternal Inheritance (Inh.), $10A^{+/-}$ dam X $10A^{+/+}$ sire) or paternally (Paternal Inh, $10A^{+/+}$ dam x $10A^{+/-}$ sire) over the course of 12 weeks on a HFD (60 kcal% fat, Ad lib feeding). P value by 2-way ANOVA with Sidak's multiple comparison. (Males: Maternal Inh. $10A^{+/-}$ n=7, Paternal Inh. $10A^{+/-}$ n=10; Females: Maternal Inh. $10A^{+/-}$ n=10, Paternal Inh. $10A^{+/-}$ n=6). (B) Lean and (C) fat body mass were normalized to body mass to calculate % Lean and % Fat mass. P value by unpaired t-test (Male: Maternal Inh. $10A^{+/-}$ n=7, Paternal Inh. $10A^{+/-}$ n=10; Female: Maternal Inh. $10A^{+/-}$ n=10, Paternal Inh. $10A^{+/-}$ n=6). (D) Body length of male mice was measured after CO₂ sacrifice. P value by unpaired t-test, *P=0.0490. (Maternal Inh. $10A^{+/-}$ n=4, Paternal Inh. $10A^{+/-}$ n=9). (E) Fasting blood glucose was measured after a 5 hour fast, via a glucometer. P value by unpaired t-test, **P=0.0081. (Male: Maternal Inh. $10A^{+/-}$ n=7, Paternal Inh. $10A^{+/-}$ n=10; Female: Maternal Inh. $10A^{+/-}$ n=6). (F) Fasting plasma insulin was measured after a 5 hour fast, P value by unpaired t-test. (Male: Maternal Inh. $10A^{+/-}$ n=6). (F) Fasting plasma insulin was measured after a 5 hour fast, P value by unpaired t-test. (Male: Maternal Inh. $10A^{+/-}$ n=6). (F) Fasting plasma insulin was measured after a 5 hour fast, P value by unpaired t-test. (Male: Maternal Inh. $10A^{+/-}$ n=6). (F) Fasting plasma insulin was measured after a 5 hour fast, P value by unpaired t-test. (Male: Maternal Inh. $10A^{+/-}$ n=6). (F) Fasting plasma insulin was measured after a 5 hour fast, P value by unpaired t-test. (Male: Maternal Inh. $10A^{+/-}$ n=6). (F) Fasting plasma insulin was measured after a 5 hour fast, P value by unpaired t-test. (Male: Maternal Inh.10



Supplemental Figure 5. *Atp10A* deletion causes changes to liver lipid metabolism in female mice after 12 weeks on a HFD. (A-G) FFAs, Total cholesterol, cholesterol esters, unesterifed cholesterol, TGs, PLs, and ceramides were measured from flash frozen livers via gas chromatography. Livers were collected after a 5 hour fast or after a 5 hour fast followed by an OGTT. P value by unpaired t-test (FFA: 10A^{+/+} n=15,10A^{-/-} n=7, *P=0.0201; Total cholesterol: 10A+/+ n=16, 10A-/- n=7; Cholesterol esters: 10A+/+ n=6,10A^{-/-} n=6; Unesterified cholesterol: 10A^{+/+} n=6,10A⁻ /- n=6; TG: 10A+/+ n=14, 10A-/- n=7; PLs: 10A+/+ n=16, 10A-/- n=7; Ceramides: 10A+/+ n=16, 10A-/- n=7). The saturation of liver (H) TG, PL, and (I) CE species was determined by gas chromatography. The fold change of unsaturated vs saturated TG, PL, CE is shown. P value by 2-way ANOVA with Sidak's multiple comparison, TG: **P=0.0025, ***P=0.0001, *P=0.0443, PL: *P=0.0297, *P=0.0281 (10A+/+ n=16, 10A-/- n=7). CE: P value by unpaired t-test, *P=0.0223 (10A^{+/+} n=6, 10A^{-/-} n=6). (J) Representative images of livers stained with H+E (5x) or Oil Red O (63x). The coalescence of the Oil Red O stain is indicated by yellow asterisks (artifact) and the arrows point to neutral lipids stained by Oil Red O. Scale bars = 10 µM. (K) Liver sections were scored using the Oil Red O Score described in the Materials and Methods. P value by nonparametric t-test (Mann-Whitney U), *P=0.0327 (10A^{+/+} n=6, 10A^{-/-} n=10). (L) The diameters of Oil Red O positive lipid droplets (LD) in the liver sections with a score of 2 were measured using ImageJ. The red line in the graph represents the SEM. P value by unpaired t-test, ****P=<0.0001 (10A+/+,120 LDs measured, n=2, 10A^{-/-}, 203 LDs measured, n=3).



Supplemental Figure 6. Validation of anti-ATP10A antibody: expression of ATP10A in HeLa cells with over-expressed HA-ATP10A and in mouse heart. (A) Table indicating the sequences of the ATP10A peptides that were injected into rabbits to produce antibodies. (B) Representative images of total cell lysates from HeLa cells with over-expressed HA-ATP10A (40 µg total protein) that were subjected to Western immunoblot analyses for expression of ATP10A using antibody produced against ATP10A peptides (left and central panels) and HA (right panel). (C) Representative immunofluorescent images of hearts from female mice showing expression of ATP10A (green) and DAPI (blue). Scale bar = 20 µm. The white asterisk indicates background staining, the white arrow indicates ATP10A positive staining, the yellow arrow indicates auto fluorescent red blood cells ($10A^{+/+}$ n=1, $10A^{-/-}$ n=1).



Supplemental Figure 7. ATP10A expression level regulates basal activity of insulin signaling pathways in endothelial cells. (A,D) Representative images and quantitative analysis of ATP10A from immunoblots of HUVEC (A) and SVEC4-10 (D) untreated (c), transfected with non-silencing siRNA or ATP10A siRNA constructs (#1, #2, #1). (B, E) Representative immunofluorescent images showing expression of ATP10A and DAPI in HUVEC (B) and SVEC4-10 (E) transfected with non-silencing siRNA (n) or ATP10A siRNA constructs (ATP10A#1, ATP10A#2, ATP10A#1). Scale bar=10 μm. (C, F) Representative images of basal phosphorylated and total IRβ, IRS-1, Akt, eNOS, GSK-3β, and cPLA2 from immunoblots of HUVEC (C) and SVEC4-10 (F) transfected with non-silencing siRNA or ATP10A#1 siRNA constructs. Note th the lane between the non-silencing and ATP10A#1 treated lanes contains the molecular weight marker.



Supplemental Figure 8. ATP10A expression regulates insulin signaling pathway in SVEC4-10 cells and mouse aorta. (A) Representative images and (B) quantitative analysis from immunoblots of phosphorylated and total IR β , IRS-1, Akt, eNOS, GSK-3 β , and cPLA₂ from SVEC4-10 cells transfected with non-silencing siRNA or ATP10A siRNA constructs and then treated with insulin for 0-60 mins. Mean measurements of 3-6 independent experiments are shown. *p≤0.05; **p≤0.005; ***p≤0.0005; ***p≤0.0001 between cells transfected with non-silencing or ATP10A siRNA by 2-way ANOVA with Sidak's multiple comparison test. (C) Representative blots and (D) quantitative analysis from immunoblots of phosphorylated and total eNOS from total aorta lysates from male mice (*10A*^{+/+} n=2, *10A*^{-/-} n=4, statistics were not done due to low n). Mean measurements of 3-6 independent experiments are shown.

Appendix B. Supplemental Tables for Chapter 2

	17 Males	15	24	21	
Avg. body mass on 12th week of HFD (g) I-test (p-value) from body mass at 12th week on HFD Avg. Age on 12th week of HFD (weeks)	24 0.7672 (ns) 10	33.9 19.4	23.6 0.0498 (*)	25.1 19.2	
Cuberr removed by ROUT analysis (C.D.Body come.	11 35.0	13	13 24 2	0 14 20.5	
t-test (p-value) from body mass at 12th week on HFD Avg. Age on 12th week of HFD (weeks) Outliers removed by ROUT analysis	0.3626 (ns) 17.4	19.5	0.0799 (ns)	19.2 0	
(E) Fasting blood glucose n Avg. body mass on 12th week of HED (g)	10 Males 26.2	11 24.2	19 23-1	14 26.5	
t-test (p-value) from body mass at 12th week on HFD Avo. Aoe on 12th week of HFD (weeks) Outliers removed by ROUT analysis	0.2418 (ns) 17.2 0	19.5 0	0.0049 (**) 0	19.2 0	
(F) Fasting plasma insulin n Avg. body mass on 12th week of HFD (g)	n Males 26.5	10 34.7	13 Eemales 23	9 20.4	
t-text (p-value) from body make at 12th week on HP-D Age on 12th week of HFD (weeks) Outliers removed by ROUT analysis	0.4579 (na)	19.5 0	16.3	19.3 0	
Avg. body mass on 12th week of HFD (g)	0 36.5	6 35.7	12 23.7	6 27.0	
Aug. Age on 12th week of HED (weeks) Outliers removed by ROUT analysis	17.5 0	19.3 0	16.3 9	19.5 0	
Figure 2: Plasma lipid and lipoproteins (A.D) Plasma EFA	10A+/+ (WT) 7 (all doge by lab kit)	10A-/- (KO) 6 (all doge by lab kit) 12	10A+/+ (WT) Eemales (11 done by Core 1 done by lab kit) 8 (7	10A-/- (KO)	04+/+ (WT): 11 fast + OGTT. 1: fast only (92% fast + OGTT. 8% fast only) 04-/- (KO), FFA: 4: fast + OGTT, 4: fast only (50% fast + OGTT, 50% fast only) 04-(KO), TG, chcl + 6 fast + OGTT, 8: fast only (02% fast + OGTT, 50% fast only)
Avg. body mass on 12th week of HFD (g) t-test (p-value) from body mass at 12th week on HFD Avg. Age on 12th week of HFD (weeks)	34.2 0.2427 (ns)	37.2	23.1 16.6 0.0014(**)	27.9	
Outliers removed by ROUT analysis (B.E) Plasma Chol n	0 Males 8 (all done by lab kit)	0 6 (all done by lab kit) 12	0 Eemales (11 done by Core, 1 done by lab kit) 12 (7	4 done by Core, 5 done by lab kit)	
Avg. body mass on 12th week of HFD (g) t-test (p-value) from body mass at 12th week on HFD Avg. Age on 12th week of HFD (weeks)	34.6 0.5880 (ns) 17.5	19.2	23.1 0.0243 (*) 16.6	26.3	
Outliers removed by ROUT analysis (C.F) Plasma TG	0 Males 9 (all done by lab kit)	0 7 (all done by lab kit) 12	0 Females (11 done by Core, 1 done by lab kit) 12 (7	0 done by Core, 5 done by lab kit)	
Avo. body mass on 12m week of HFD (a) t-test (p-value) from body mass at 12m week on HFD Avg. Age on 12m week of HFD (weeks) Outliers removed by 2010 analysis	0.2128 (ns) 17.6	19.1	24.1 0.0243 (*) 16.6	19.3	
(G-I) Licoprotein fractionation	N/A N/A	N/A N/A	G Females, choi 23.0	6 29.2	
t-test (p-value) from body mass at 12th week on HFD Avg. Age on 12th week of HFD (weeks) Outliers removed by ROUT analysis	N/A N/A	N/A N/A	0.0274 (*) 16.3 N/A	19.3 N/A	
D Avg. body mass on 12th week of HFD (g)	Malos N/A N/A	N/A N/A	G Eemales. TG	7 20.6	
t-test (o-value) from body mass at 12th week on HED Avg. Age on 12th week of HED (weeks) Outliers removed by ROUT analysis	N/A N/A	N/A N/A	16.3 0.0372 (*) N/A	19.3 N/A	
Figure 4: Plasma lipidomics and VF RNASeg (A.B) Plasma lipidomics	10A+/+ (WT) Males	<u>10A-/- (KO)</u>	10A+/+ (WT) Eemalez	<u>10A-/- (KQ)</u>	
Avg. body mass on 12th week of HFD (g) t-test (p-value) from body mass at 12th week on HFD to any one of 12th week of HFD (weeks)	N/A N/A	N/A Indui II	22.9 16.5 0.0068 (**)	28.4	7
Outliers removed by ROUT analysis (C) VF RNASeq	N/A Males	N/A N/A	N/A 2	N/A 1	0A+/+ (WT): 2: fast + OGTT, 1: fast only (67% fast + OGTT, 33% fast only) 0A+/- (KO): 1: fast + OGTT, 2: fast only (22% fast + OGTT, 67% fast only)
Ava. body mass on 12th week of HED (o) t-test (p-value) from body mass at 12th week on HED Ava, Aos on 12th week of HED (weeks)	N/A N/A	N/A N/A	23.3 0.0261 (*) 17.3	21.5 19.2	
Outliers removed by ROUT analysis Figure 5: Liver signaling (WP analysis)	N/A 10A+/+ (WT)	N/A 10A-/- (KO)	N/A 10A+/+ (WT)	N/A 10A-/- (KO)	
Aug. body mass on 12th week of HFD (g)	N/A Males N/A	N/A N/A	7 Eemalex 22.9	7 27.6	0A+/+ (WT): 2: fast + OGTT, : 5 fast only (29% fast + OGTT, 71 % fast only) 0A-/- (KO): : 4 fast + OGTT, : 3 fast only (57% fast + OGTT, 43% fast only)
Ltest (p-value) from body mass at 12th week on HFD Avg. Age on 12th week of HFD (weeks) Outliers removed by ROUT analysis	N/A N/A	N/A N/A	16.7 0	19.1 0	
Avg. body mass on 12th week of HFD (g)	N/A N/A	N/A N/A	23	0 1 27.3	0A+/+ (WT): 2: fast + OGTT, : 6 fast only (25% fast + OGTT, 75% fast only) 0A-/- (KO): : 4 fast + OGTT, : 4 fast only (50% fast + OGTT, 50 % fast only)
Aug. Age on 12th week of HED (weeks) Outliers removed by ROUT analysis	N/A N/A	N/A N/A	16.9 0	19.1 0	
Figure 5: 10A Expression (WB & IF) (A) 10A Expression in Mouse tissues (WB)	10A+/+ (WT) 2 (yas def beat:1 testes: 1) 2 (10A-/- (KO)	10A+/+ (WT) Eemales (fed HED for 12: 1 (brain, liver, VE)	10A-/- (KO) weeks) 1 (brain liver VE)	HC: n=tissues from different mice imaged in the same session where the representative image was image
Body mass at sacrifice (g) t-test (p-value) from body mass at 12th week on HFD Age (weeks)	N/A N/A 7.5	0 N/A	27 N/A 19	25	
Outliers removed by ROUT analysis (B) 10A Expression in Testes & Vas deferens (IF) n	N/A Males	N/A 1	N/A Females	N/A N/A	
Body mass at sacrifice (g) t-test (p-value) from body mass at 12th week on HFD Age (weeks)	26 N/A	25	N/A N/A	N/A N/A	
(C) 10A Expression in Liver n (C) 10A Expression in Liver n function of the state of MED (c)	N/A Males	N/A N/A	N/A Females (Liver)	1	
t-test (p-value) from body mass at 12th week on HFD Avg. Age on 12th week of HFD (weeks) Outliers removed by ROUT analysis	N/A N/A	N/A N/A	18.7 N/A 18.7 N/A	21 N/A	
(C) 19A Expression in VE n Avg. body mass on 12th week of HFD (g)	N/A N/A	N/A N/A	3 24	2	
t-test (p-value) from body mass at 12th week on HFD Avg. Age on 12th week of HFD (weeks) Outliers removed by ROUT analysis	N/A N/A	N/A N/A	0.7345 (ns) 18.7 N/A	19.5 N/A	
Figure 7: EC signaling	10A+/+ (WT)	10A-/- (KO)	104+/+ (WT)	10A-/- (KO)	
(E.F) Mouse Aorta	Males.	51/4	Eemales (all normal ch	new)	
(E-F)4 mouse at acata Avg. Body mouse at acata Avg. Body mouse at acata to a set of the set o	N/A N/A N/A	N/A N/A	5 23.0 20.4 Eemales (all normal ch 23.0 0.5209 (ns)	1000) 4 22.0 17.0	
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	Pathway Kego	1					
<u>geneSet</u>	description	size	overlap	expect	enrichmentRatio	pValue	FDR
g.jp/kegg-bin/show_pathway? mmu04972+13487+1878	Pancreatic secretion	103	5	0.809063	6.179985	0.0012387	0.393894
tp://www.kegg.jp/kegg-bin/show_pathway? mmu00730+	Thiamine metabolism	15	2	0.117825	16.974359	0.0059725	0.566786
g-bin/show pathway? mmu04080+110902+11555+126	Neuroactive ligand-receptor interaction	289	7	2.270091	3.083577	0.0072516	0.566786
www.kegg.ip/kegg-bin/show_pathway?mmu05150+1351	Staphylococcus aureus infection	56	3	0 439879	6 820055	0.0095348	0.566786
www.kegg.ip/kegg-bin/show_pathway? mmu00561+2355	Glycerolipid metabolism	61	3	0 479154	6 261034	0.0120409	0.566786
tp://www.kegg.ip/kegg-bin/show_pathway2_mmu0/96/+	Proximal tubule bicarbonate reclamation	22	2	0 17281	11 573/27	0.0126837	0.566786
ip.//www.kegg.jp/kegg-bin/show_pathway? mindo4304	Phagocomo	101	5	1 421752	2 516797	0.0125901	0.566786
g.jp/kegg-bill/silow_patitway? mindo4143+12643+1498	Filagosoffie	101	0	0.00400	0.700000	0.0133001	0.500780
p://www.kegg.jp/kegg-bin/snow_pathway? mmu00790+1	Folate biosynthesis	20	2	0.20423	9.792899	0.0174897	0.566786
www.kegg.jp/kegg-bin/snow_pathway?mmu04976+1384	Bile secretion	/1	3	0.557704	5.379198	0.018093	0.566786
)://www.kegg.jp/kegg-bin/show_pathway? mmu00650+2	Butanoate metabolism	27	2	0.212085	9.430199	0.0187942	0.566786
Significantly di	<u>ferential expressed genes in Pancreat</u>	<u>ic secretio</u>	<u>n</u>				
Stable IDs	Feature_gene_name	baseMean	pvalue	padj	FoldChange		
ENSMUSG0000041193.15	Pla2g5	327.6929	0	0	21.6047666		
ENSMUSG0000007107.6	Atp1a4	258.5906	0	0.000001	4.8748642		
ENSMUSG0000071553.10	Cpa2	196.022	0	1.2E-06	3.7086972		
ENSMUSG0000020431.5	Adcy1	104.9107	0.00022	0.020501	0.3598145		
ENSMUSG0000001225.12	Slc26a3	29.11604	0.00024	0.021209	3.9715976		
Significantly dif	ferential expressed genes in Thiamine	metabolis	<u>m</u>				
Stable IDs	Feature gene name	baseMean	pvalue	padj	FoldChange		
ENSMUSG0000026817.14	Ak1	1358.032	0.000292	0.023143	2.018769		
ENSMUSG0000028766 10	Alpl	237 0382	0.000518	0 034247	2 843773		
	7.101	201.0002	0.000010	0.004241	2.040110		
Significantly differential	expressed genes in Neuroscius ligan	d-recentor	Interaction				
Stable IDc	Easture gang name	hacoMoan	nyaluo	nadi	FoldChange		
<u>Stable IDS</u>		56 43242	1 47E 05	0.002950	571 2601672		
	Gprou	50.43242	1.47E-05	0.003859	5/1.2601672		
ENSMUSG0000040495.4	Chrm4	14.01271	2.69E-05	0.005626	25.0694979		
ENSMUSG0000043017.9	Ptgir	400.5867	5.13E-05	0.008149	2.345606		
ENSMUSG0000044338.9	Aplnr	803.3216	0.00076	0.04297	2.7616563		
ENSMUSG0000021478.6	Drd 1	52.58807	0.0009	0.048217	0.2768258		
ENSMUSG0000022041.10	Chma2	169.0929	0.000918	0.048854	2.1544465		
ENSMUSG0000045730.4	Adrb2	637.536	0.000944	0.049661	0.4256661		
Significantly different	ial expressed genes in Staphylococcu	<mark>s aureus ir</mark>	nfection				
Significantly different Stable IDs	ial expressed genes in Staphylococcu Feature gene name	<mark>s aureus ir</mark> baseMean	n <u>fection</u> pvalue	padj	FoldChange		
Significantly different Stable IDs ENSMUSG0000069441.3	<mark>ial expressed genes in Staphylococcu</mark> Feature gene name Dsg1a	<mark>s aureus ir</mark> baseMean 61.66929	<mark>ifection</mark> pvalue 4E-07	<u>padi</u> 0.000233	FoldChange 5.596025		
Significantly different Stable IDs ENSMUS G00000069441.3 ENSMUS G00000061928.6	<mark>ial expressed genes in Staphylococcu Feature gene name Dsg1a Dsg1b</mark>	<mark>s aureus ir</mark> baseMean 61.66929 20.82076	n <u>fection</u> pvalue 4E-07 2.61E-05	<u>padi</u> 0.000233 0.00562	FoldChange 5.596025 19.302742		
<u>Significantly different</u> <u>Stable IDs</u> ENSMUS G0000069441.3 ENSMUS G00000061928.6 ENSMUS G00000026580.16	<mark>ial expressed genes in Staphylococcu</mark> Feature gene name Dsg1a Dsg1b Selo	<mark>s aureus ir</mark> baseMean 61.66929 20.82076 210.5568	nfection pvalue 4E-07 2.61E-05 0.00073	padi 0.000233 0.00562 0.04214	FoldChange 5.596025 19.302742 2.465355		
Significantly different Stable IDs ENSMUSG0000069441.3 ENSMUSG00000061928.6 ENSMUSG00000026580.16	<mark>ial expressed genes in Staphylococcu</mark> Feature gene name Dsg1a Dsg1b Selp	<mark>s aureus ir</mark> baseMean 61.66929 20.82076 210.5568	nfection pvalue 4E-07 2.61E-05 0.00073	<u>padi</u> 0.000233 0.00562 0.04214	FoldChange 5.596025 19.302742 2.465355		
Significantly different Stable IDs ENSMUSG0000069441.3 ENSMUSG0000061928.6 ENSMUSG0000026580.16	ial expressed genes in Staphylococcu Feature gene name Dsg1a Dsg1b Selp	<mark>s aureus ir</mark> baseMean 61.66929 20.82076 210.5568	nfection pvalue 4E-07 2.61E-05 0.00073	padi 0.000233 0.00562 0.04214	FoldChange 5.596025 19.302742 2.465355		
Stable IDs Stable IDs ENSMUS G00000069441.3 ENSMUS G00000061928.6 ENSMUS G00000026580.16 Stable IDs Stable IDs	ial expressed genes in Staphylococcu Feature gene name Dsg1a Dsg1b Selp rential expressed genes in Glycerolip	s aureus ir baseMean 61.66929 20.82076 210.5568 d metaboli	nfection pvalue 4E-07 2.61E-05 0.00073 sm	padi 0.000233 0.00562 0.04214	FoldChange 5.596025 19.302742 2.465355		
Significantly different Stable IDs ENSMUSG0000069441.3 ENSMUSG00000061928.6 ENSMUSG00000026580.16 Significantly diffe Stable IDs ENSMUSC0000024570.5	ial expressed genes in Staphylococcu Feature gene name Dsg1a Dsg1b Selp rential expressed genes in Glycerolip Feature gene name	s aureus ir baseMean 61.66929 20.82076 210.5568 d metaboli baseMean	Infection pvalue 4E-07 2.61E-05 0.00073 sm pvalue 0	padi 0.000233 0.00562 0.04214 padi	FoldChange 5.596025 19.302742 2.465355 FoldChange		
Significantly different Stable IDs ENSMUS G0000069441.3 ENSMUS G00000061928.6 ENSMUS G00000026580.16 Significantly different Stable IDs ENSMUS G00000024768.5	ial expressed genes in Staphylococcu Feature gene name Dsg1a Dsg1b Selp rential expressed genes in Glycerolip Feature gene name Lipf	s aureus ir baseMean 61.66929 20.82076 210.5568 d metaboli baseMean 231.5996	Infection pvalue 4E-07 2.61E-05 0.00073 sm pvalue 0	padi 0.000233 0.00562 0.04214 padi 0	FoldChange 5.596025 19.302742 2.465355 FoldChange 16.1785033		
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Significantly different Stable IDs ENSMUS G00000069441.3 ENSMUS G00000061928.6 ENSMUS G00000026580.16 Significantly diffe Stable IDs ENSMUS G00000024768.5 ENSMUS G00000024768.5 ENSMUS G00000024768.5 ENSMUS G00000024768.5 ENSMUS G00000024768.5	ial expressed genes in Staphylococcu Feature gene name Dsg1a Dsg1b Selp rential expressed genes in Glycerolip Feature gene name Lipf Mogat1 Glyctk	s aureus ir baseMean 61.66929 20.82076 210.5568 d metaboli baseMean 231.5996 674.7383 130.4926	Infection pvalue 4E-07 2.61E-05 0.00073 sm pvalue 0 0.000242 0.000281	padi 0.000233 0.00562 0.04214 padi 0 0.021213 0.022637	FoldChange 5.596025 19.302742 2.465355 9.302742 2.465355 9.302742 2.465355 9.302742 2.465355 9.302742 10.1785033 0.4207309 2.285067		
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Appendix C: Supplemental Figures for Chapter 3

А			ATP10A Peptide 1	ATP10A Peptide 3					
	hATP10A	RRRRRE	GRTRT-VRSNLLP	PGAEDPAAGAAKGERR	RRRGC	55			
	rATP10A	IOAAGRAGRARCAPNCCRPPGAEDSAAGAAKGERRPRRRLRGC							
	mATP10A	RRPRRRWEG <mark>RTRT-VRSNLLP</mark> PLG <mark>TEDSTIGAPKGER</mark> LLMRGC							
	hATP10B	HRWQWRVRDGFPHCPSETTPLLSPEKGRQSYNLTQQRVVFPNNSIFHQDWEEV							
	mATP10B	LRWKWRTQDGFSQSPSETTPLLSPETDRQSHNTAEQRVVYPNNSMCHQDWKKV							
1	mATP10D	ARHHWRRLSHGRAQGEDERPYNYASLLACGGKSSRTPRPAGKHRVVIPHLOCFKDEYERF 1							
	hATP10D	ARYHWRRLI	RGATRDDDSGPYNYSSLLA	ACGRKSSQTPKLSGRHRIV	VPHIQPFKDEYEKF	67			
				ATP10A Peptide	5				
	hATP10A	G-SVSORGS	IGSH0	SVRVVHRTOSTKSHRRTG	SRAEAKRASMLSKH	520			
	rATP10A	N-TVSORGS	IGSH0	SIRIMSRSOSTRSHRRTG	SRAEARRASILSKH	512			
i i	mATP10A	G-TISHRGS	TGSHQ	SIWMTHKTQSIKSHRRTG	SRAEAKRASMLSKH	524			
	hATP10B	CLSFSARWA	QDPATMRSQKGAQPLRRSQ	SARVPIQGHYR	QRSMGHR-ESSQPP	533			
	mATP10B CLSFPPRWAOGSTTMRSOGGAOPLRRCHSARVPIOSHCRORSVGRW-ETSOPP 5								
	mATP10D	GGSLSNMAR	PRAQGCRT-VPSGPLGK-F	SAQLSGSTSAV	GNGEGSGEVPHSRQ	590			
	hATP10D	SGSLSNMAK	PRAPSCRT-VHNGPLGNKE	SNHLAGSSFTL	GSGEGASEVPHSRQ	538			
В									
	ATP10A	Peptides	1	3	5				
	Amino acid number 27-38 41-53 498-509								
	Amino sequ	o acid ence	RTRTVRSNLLPP	TEDSTIGAPKGER	HKTQSIKSHRRT				



Supplemental Figure 9. Validation of ATP10A antibody: expression of ATP10A in HeLa cells with over-expressed HA-ATP10A. (A) Alignments of human (h), rat (r), and mouse (m) ATP10A, ATP10B, and ATP10D created using Clustal Omega with the ATP10A peptides that were chosen for antibody production highlighted. These peptides were chosen based on conservation between mouse and human ATP10A orthologs and lack of conservation with ATP10B and ATP10D paralogs. (B) Table indicating the sequences of the ATP10A peptides that were injected into rabbits to produce antibodies, peptides are color-coded according to the colors of the peptides in (A). (C,D) Representative images of total cell lysates from HeLa cells with (+) or without (-) overexpressed (OE) human HA-ATP10A (40 µg total protein) that were subjected to Western immunoblot analyses for expression of ATP10A using antibody produced against (C) ATP10A peptides or (D) HA. Peptides are color-coded according to the colors of the peptides in (A).



Supplemental Figure 10. Reintroduction of *Atp10D* does not rescue infertility, smaller testes, or low sperm cell count in Atp10A deficient mice. (A) Sequencing was performed to validate the genotype of the Atp10D mice. Briefly, CRISPR was used to replace the premature stop codon found in the Atp10D sequence in C57BL/6J mice (*, *Atp10D*^{-/-} mice) to glutamine that is found in other mouse strains (Q, *Atp10D*^{+/+} (corrected) mice). The underlined nucleotides indicate CRISPRmediated changes to the DNA sequence, for the purposes of changing the amino acid or introducing restriction digest cut sites for genotyping purposes. (B) Mating studies were done by housing pairs of mice together (without separating) for approximately 9 weeks and recording the number of litters produced. (See supplemental table 1 for more details). (C) The wet mass of testes was measured in 14-21-week-old mice, ***P=0.0002. (D) Sperm cells collected from the caput epididymis were counted using a hemocytometer, ****P<0.0001. (E) % Sperm cell motility was measured using the equation: motile sperm/total sperm counted. P-value by unpaired t-test.



Supplemental Figure 11. *Atp10A* deficiency results in changes to signaling in the vas deferens. Total tissue lysates from vas deferens from the $10A^{+/+}$ and $10A^{-/-}$ mice were subjected to Western immunoblot analysis. (A) Representative blots for total and/or phosphorylated ERK1/2, p38, Akt, and cPLA₂. (B-E) The phosphoproteins were normalized to their respective total protein, β -actin levels, and total protein from $10A^{+/+}$ samples. Mean measurements of 4-6 independent experiments are shown. P value by unpaired t-test. ($10A^{+/+}$ n=7, $10A^{-/-}$ n=7, B: *P=0.0313, C: **P= 0.0024, D: ****P<0.0001, E: *P=0.0376).

Appendix D. Supplemental Table for Chapter 3

Figure 9 (all 10D -/-)	<u>10A+/+ (WT)</u>	<u>10A-/- (KO)</u>		
(A) Vas deferens WB				
n	1	1		
Body mass at sacrifice (g)	did not measure	did not measure		
Age at sachlice (weeks)	/ N/A	0 N/A		
(B) Testes WB	nva -	10/5		
n	1	1		
Body mass at sacrifice (g)	23	24		
Age at sacrifice (weeks)	8	8		
Outliers removed by ROUT analysis	N/A	N/A		
(C) vas deferens IF	1	2		
Avg. body mass at sacrifice (g)	26	28.5		
Avg. age at sacrifice (weeks)	11	15		
Outliers removed by ROUT analysis	N/A	N/A		
<u>(D) Testes IF</u>				
n	2	2		
Avg. body mass at sacrifice (g)	27.5	28.5		
Avg. age at sachlice (weeks)	17 N/A	17 N/A		
Oddiela temoved by 1000 Faharysia	10/5	10/6	_	
(E) Mating studies	Red text= S	ire is 10A -/-	1	
Order: Sire (M) x Dam (F)	Average time parents h	noused together (weeks)	Average pups per litter	
10A +/+ x 10A +/+ (1 pair)	21	0.9	6	
10A +/+ x 10A -/- (2 pairs)	6	.6	5	
10A -/- x 10A +/+ (3 pairs)	9	1.8	N/A	
10A +/- x 10A +/- (1 pair) 10A -/- x 10A +/- (3 pairs)	1	5.9	85	
10A +/- x 10A +/- (5 pairs)	1	2	7.1	
10A -/- x 10A -/- (3 pairs)	1	0.9	N/A	
Figure 10 (all 10D -/-)	<u>10A+/+ (WT)</u>	<u>10A+/- (Het)</u>	<u>10A-/- (KO)</u>	
(A) Testes mass (mg), (B), Sperm cells/mL, (C) % sperm cell motility				
n Aug hadu maaa at aanifaa (g)	9	2	14	
Avg. bouy mass at sacrifice (g)	20.8	20	20.7	
Outliers removed by ROUT analysis	0	0	0	
Oddiela temoved by NOOT analysis	5	0	ő	
Figure 11 (all 10D -/-)	10A+/+ (WT)	10A-/- (KO)		
(C) Seminiferous tubule scoring				
n	6	12		
Avg. body mass at sacrifice (g)	26	26.8		
Avg. age at sacrifice (weeks)	12.2	11.6		
Outliers removed by ROUT analysis	N/A	N/A		
Figure 12 (all 10D -/-)	10A+/+ (WT)	10A-/- (KO)		
(B) Plasma T		· · · · · · · · · · · · · · · · · · ·		
n	16	18		
Avg. body mass at sacrifice (g)	33.5	33.8		
Avg. age at sacrifice (weeks)	14.1	15.4		
Outliers removed by ROUT analysis	3 10A+/+ (M/T)	1 10A-(- (KO)		
<u>(c) Ln</u>	<u>104+/+ (WI)</u>	<u>104 (KO)</u>		
Ν	11	17		
Avg. body mass at sacrifice (g)	26	27		
Avg. age at sacrifice (weeks)	11.5	11.1		
Outliers removed by ROUT analysis	0	1		
(D) FSH	<u>10A+/+ (WI)</u>	<u>10A-/- (KO)</u>		
n	11	18		
Avg. body mass at sacrifice (g)	26	27		
Avg. age at sacrifice (weeks)	11.5	11.1		
Outliers removed by ROUT analysis	1	0		
(E) Inhibin B	<u>10A+/+ (WT)</u>	<u>10A-/- (KO)</u>		
	11	15		
Avg. bouy mass at sacrifice (g)	20	27.3		
Outliers removed by ROUT analysis	0	0		
<u>Figure 13 (Testes, all 10D -/-)</u>	<u>10A+/+ (WT)</u>	<u>10A-/- (KO)</u>		
	6-8	6-8		
Avg. body mass at sacrifice (g)	27.4	28		
Avg. age at sachlice (weeks) Outliers removed by ROUT analysis	0	14.1 LHR: 1. pAkt: 1		
		criter, pricer		
Supplemental Figure 10 (All 10D +/+)				
(A) Mating studies	Red text= S	ire is 10A -/-		
Order: Sire (M) x Dam (F)	Average time parents h	noused together (weeks)	Average pups per litter	
10A +/+ x 10A -/- (2 pairs)	8	.9	5.8 N/A	
(BCD) Testes mass Sperm collocation coll motility	104+/+ 100 +/+	104-6 100 ±4	N/A	
	100.01, 100 101	<u>100-7-, 100 1/1</u>		
n	4	5		
Avg. body mass at sacrifice (g)	29.8	29.4		
Avg. age at sacrifice (weeks)	18.5	17.8		
Outliers removed by ROUT analysis	0	0		
Supplemental Figure 44 (Vec deference all 400 (104.1/1 (1477)	104 / (KO)		
Supplemental Figure 11 (Vas deferens, all 10D -/-)	1UA+/+ (WI)	<u>10A-/- (KO)</u>		
n				
	4-7	4-7		
Avg. body mass at sacrifice (g)	4-7 15.4	4-7 15		
Avg. body mass at sacrifice (g) Avg. age at sacrifice (weeks)	4-7 15.4 27.9	4-7 15 28.6		
Avg. body mass at sacrifice (g) Avg. age at sacrifice (weeks) Outliers removed by ROUT analysis	4-7 15.4 27.9 0	4-7 15 28.6 0		





Supplemental Figure 12. Metabolic parameters in the 10A Deficient, 10D Correction mouse model (A, B) Weight gain of (A) male and (B) female $10A^{+/+}10D^{+/+}$ and $10A^{-/-}10D^{+/+}$ over the course of 12 weeks on a HFD (60 kcal% fat, Ad lib feeding). P value by 2-way ANOVA with Sidak's multiple comparison. (C) Lean and (D) fat body mass were normalized to the sum of lean mass and fat mass to calculate % Lean and % Fat mass ($10A^{+/+}10D^{+/+}$ n=4, $10A^{+/+}10D^{-/-}$ n=3). (E) Fasting blood glucose was measured after a 5 hour fast, via a glucometer ($10A^{+/+}10D^{+/+}$ n=3, $10A^{+/+}10D^{-/-}$ n=3). (C-E) P value by unpaired t-test. Average body mass (g) at end of HFD: Males: $10A^{+/+}10D^{+/+}$: 36 g, $10A^{-/-}10D^{+/+}$: 33.3 g, Females: $10A^{+/+}10D^{+/+}$: 24.5 g, $10A^{+/+}10D^{+/+}$: 29 g

Appendix F: Supplemental Tables for Chapter 6

	Primer sequence (5' to 3')						
Target	<u> </u>	<u>R</u>					
Pla2g5	TGG GGT TCT GAG ATG TCA CT	TCT TCC TCC TGT CTT GTT GC					
SCD1	CTGTACGGGATCATACTGGTTC	GCCGTGCCTTGTAAGTTCTG					
LPL	TGG CAT AAG TCA GGT CCA TT	GAG CCA TGT CTT CAA CTG CT					
Pla2g6	ATC CAC ACA GCC ATG AAG TT	TGT TCC CTG AGG AGC TAG TG					
FAS	GCGATGAAGAGCATGGTTTAG	GGCTCAAGGGTTCCATGTT					
DGAT1	CCA GGT GGT GTC TCT GTT TC	AGA TGA TTG TGG CCA GGT TA					
DGAT2	CTGTGCTCTACTTCACCTGGCT	CTGGATGGGAAAGTAGTCTCGG					
PPARa	GCCTGTCTGTCGGGATGT	GGCTTCGTGGATTCTCTTG					
PPARg	GCCCTTTGGTGACTTTATGGA	GCAGCAGGTTGTCTTGGATG					
LXRa	CCGACAGAGCTTCGTCC	CCCACAGACACTGCACAG					
Srebpc1	CGCGGAAGCTGTCGGGGTAG	AAATGTGCAATCCATGGCTCCGTGGTC					
CGI58	GGT TAA GTC TAG TGC AGC	AAG CTG TCT CAC CAC TTG					
	cca ggg aga tgc tct gtg ttc	acc cag aca gcg ctc ttt gat					
Cyp7a1	AGCAACTAAACAACCTGCCAGTAC TA	GTCCGGATATTCAAGGATGCA					
Cyp8b1	GCCTTCAAGTATGATCGGTTCCT	GATCTTCTTGCCCGACTTGTAGA					
Hprt1 (housekeeping gene)	GCTGACCTGCTGGATTACAT	TTGGGGCTGTACTGCTTAAC					

Supplemental Table 4

			Visceral Fat, Females, 12	weeks after HFD feedi	ng			
	qPCR results					A sequencing results	(10A+/+ n= 3)	, <i>10A-/-</i> n=3)
	Sample	size (n)						
Target	<u>10A+/+</u>	<u>10A-/-</u>	p-value (unpaired t-test of Relative mRNA level (2^-ΔΔCq))	Significant (Y or N)	Target	log2FoldChange	p-value	Significant (Y or N)
Pla2g5	2	4	0.2526	Ν	Pla2g5	4.433277742	2.03E-17	Y
SCD1	2	4	0.8275	Ν	SCD1	0.355620653	0.242335	N
LPL	3	4	0.7678	Ν	LPL	0.294114678	0.123495	N
Pla2g6	3	4	0.8143	Ν	Pla2g6	0.234601663	0.309316	N
FAS	3	4	0.9077	Ν	FAS	-0.099676899	0.588441	N
DGAT1	3	4	0.6299	Ν	DGAT1	0.017153228	0.933198	N
DGAT2	3	4	0.6174	Ν	DGAT2	0.439107779	N/A	N/A
PPARa	2	4	0.3831	Ν	PPARa	not found in data		
PPARg	3	4	0.3658	Ν	PPARg	not found in data		
LXRa	3	4	0.824	Ν	LXRa	not found in data		
SREBP-1C	2	4	0.824	Ν	Srebpc1	not found in data		
CGI58	2	4	0.824	Ν	CGI58	not found in data		
		Liver	qPCR Results, Females, 12 weeks after HFD feeding					
	Sample	size (n)						
Target	<u>10A+/+</u>	<u>10A-/-</u>	p-value (unpaired t-test of Relative mRNA level (2^-ΔΔCq))	Significant (Y or N)				
PPARg	4	4	0.0474	Y *				
FAS	4	3	0.0067	Y **				
SCD1	4	5	0.4074	Ν				
Cyp7a1	4	4	0.7601	N				
Cyp8b1	4	4	0.1477	N				
LPL	4	3	0.2685	N				