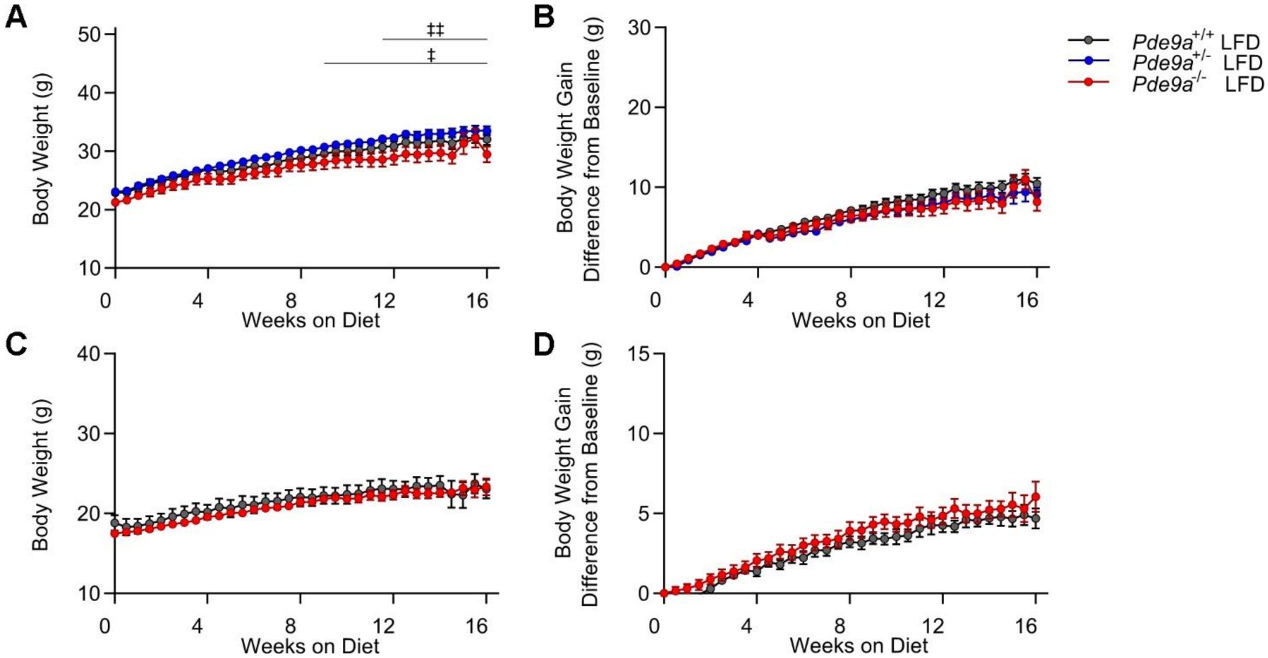
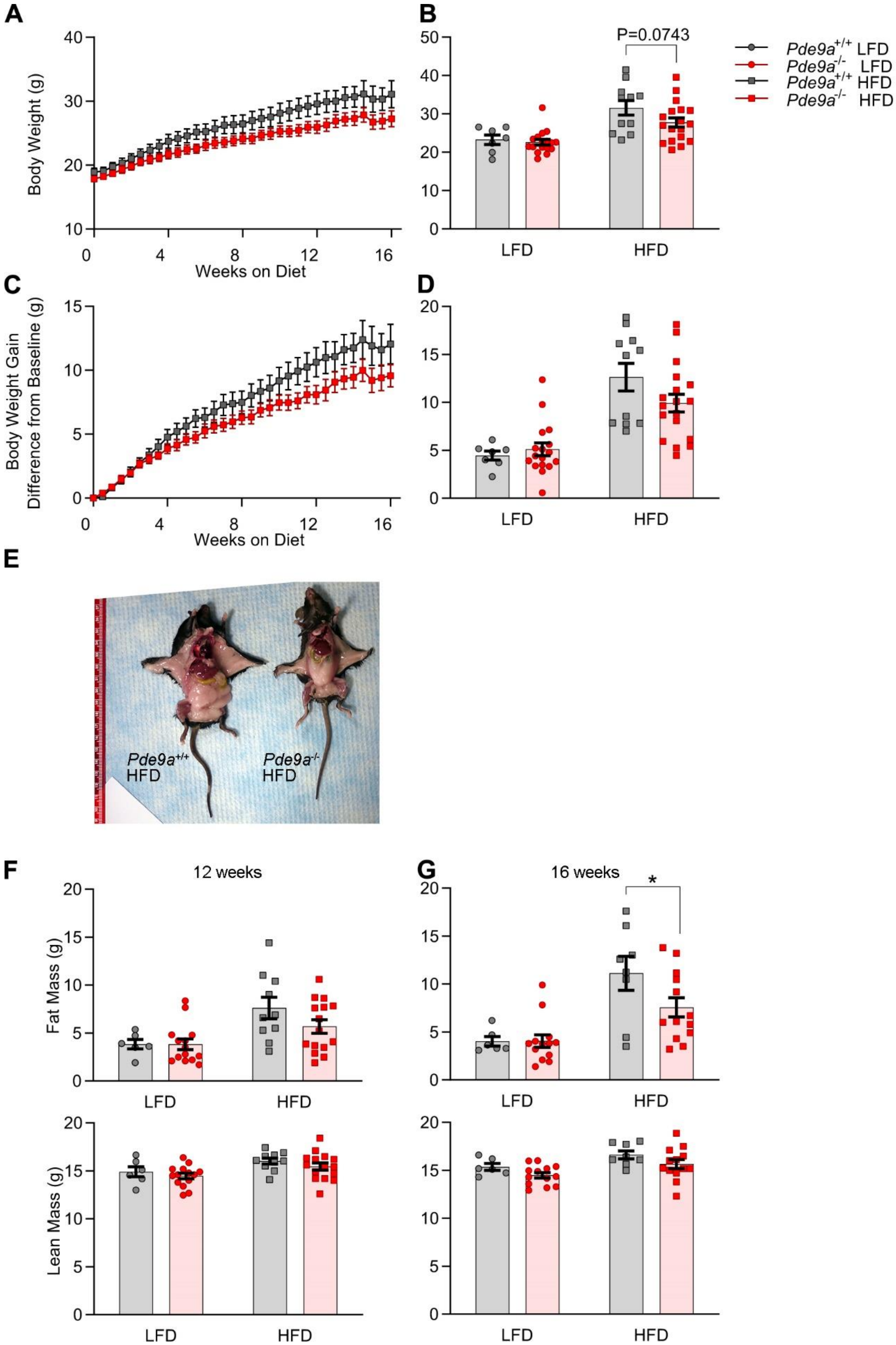
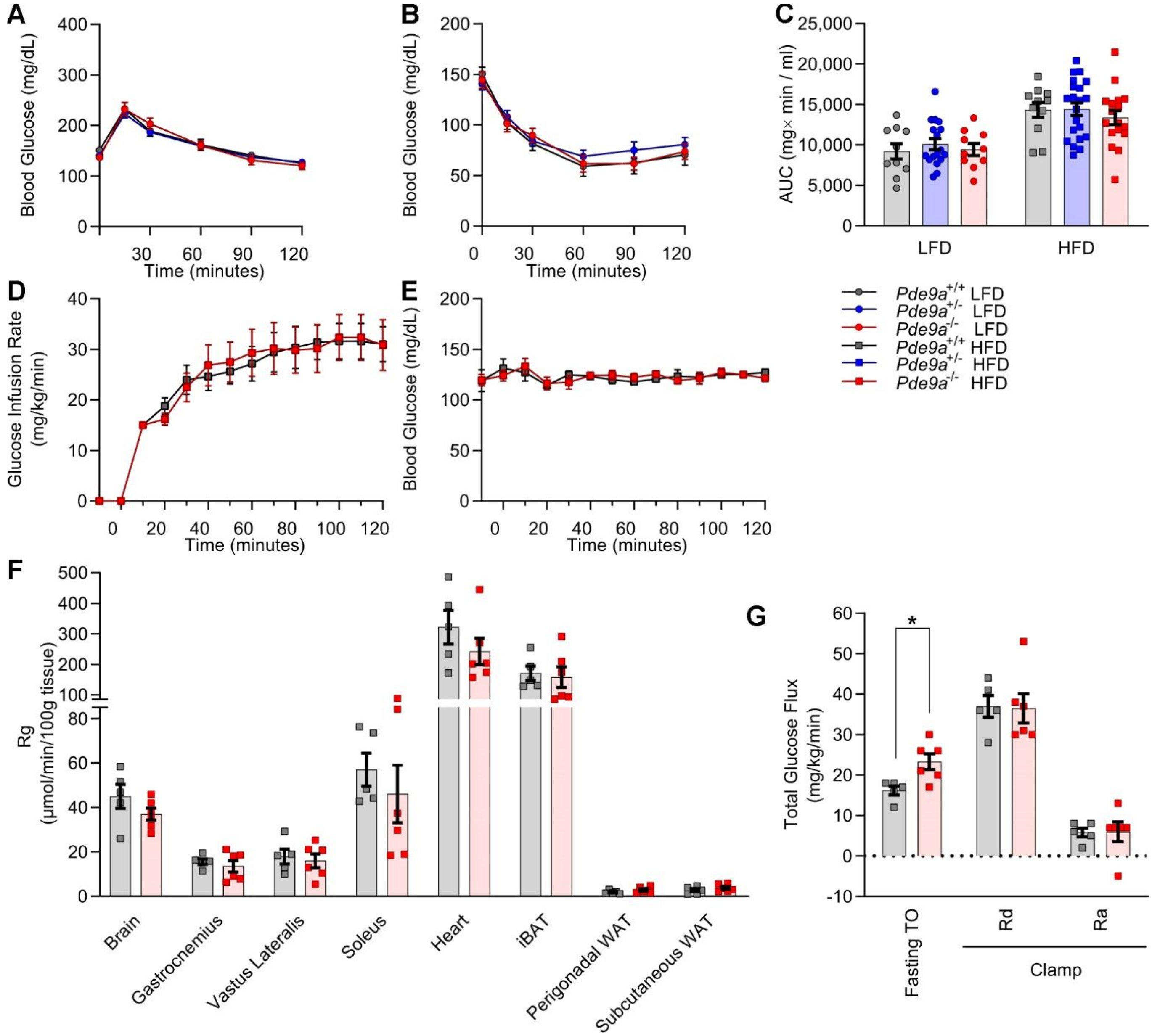


Supplemental Figure S1. Low fat diet fed *Pde9a*$^{-/-}$ mice have no difference in body weight.
(A) Male *Pde9a*$^{+/+}$, *Pde9a*$^{+/-}$, and *Pde9a*$^{-/-}$ mice were fed a LFD for 16 weeks beginning at 6 weeks of age. (B) Body weight gain. (C) Female *Pde9a*$^{+/+}$ and *Pde9a*$^{-/-}$ mice were fed a LFD for 16 weeks beginning at 6 weeks of age. (D) Body weight gain. For (A-B) N = 10 *Pde9a*$^{+/+}$ LFD, 17 *Pde9a*$^{+/-}$ LFD, 11 *Pde9a*$^{-/-}$ LFD. For (C-D), N = 7 *Pde9a*$^{+/+}$ LFD, 17 *Pde9a*$^{-/-}$ LFD. Data were analyzed by 2-way ANOVAs with repeated measures. Post-hoc analyses were performed using Sidak's multiple comparisons test for *Pde9a* genotype only and are indicated on figures with ‡ comparing *Pde9a*$^{+/-}$ vs. *Pde9a*$^{-/-}$. * or ‡, $P < 0.05$; ** or ‡‡, $P < 0.01$.



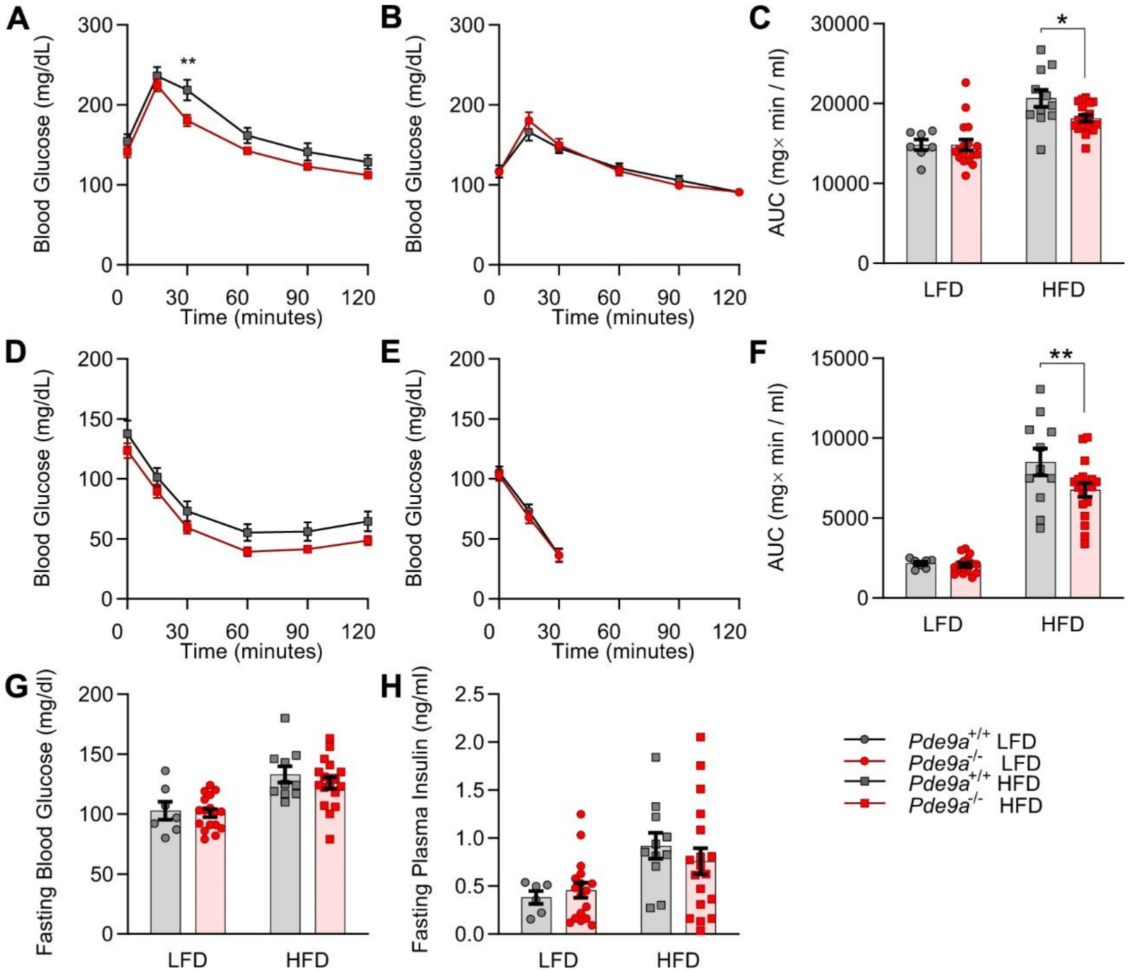
A
Body Weight (g)
Weeks on Diet
B
P=0.0743
LFD
HFD
Pde9a+/+ LFD
Pde9a-/- LFD
Pde9a+/+ HFD
Pde9a-/- HFD
C
Body Weight Gain
Difference from Baseline (g)
Weeks on Diet
D
LFD
HFD
E
Pde9a+/+
HFD
Pde9a-/-
HFD
F
12 weeks
Fat Mass (g)
Lean Mass (g)
LFD
HFD
G
16 weeks
*
LFD
HFD

Supplemental Figure S2. Female *Pde9a*$^{-/-}$ mice exhibit a modest protection from high fat diet induced obesity. (A) Female *Pde9a*$^{+/+}$ and *Pde9a*$^{-/-}$ mice were fed a LFD or HFD for 16 weeks beginning at 6 weeks of age. LFD results are in Supplemental Figure S1. (P = 0.0003, genotype × time interaction for HFD) (B) Terminal body weight. (C) Body weight gain. LFD results are in Supplemental Figure S1. (P = 0.0003, genotype × time interaction for HFD) (D) Cumulative body weight gain. (E) Representative images of *Pde9a*$^{+/+}$ and *Pde9a*$^{-/-}$ littermates that were fed HFD. Body composition at 12- (F) and 16-weeks (G) of HFD feeding. Data are mean ± SEM. Analyses were performed using 2-way ANOVA. For (A) and (C), 2-way ANOVAs were performed with repeated measures. Post-hoc analyses were performed using Sidak's multiple comparisons test for *Pde9a* genotype only and are indicated on figures with * $P < 0.05$ comparing *Pde9a*$^{+/+}$ vs. *Pde9a*$^{-/-}$. For (A-D), N = 7 *Pde9a*$^{+/+}$ LFD, 17 *Pde9a*$^{-/-}$ LFD, 11 *Pde9a*$^{+/+}$ HFD, 18 *Pde9a*$^{-/-}$ HFD. For (F-G), N = 6 *Pde9a*$^{+/+}$ LFD, 13-14 *Pde9a*$^{-/-}$ LFD, 8-10 *Pde9a*$^{+/+}$ HFD, 13-15 *Pde9a*$^{-/-}$ HFD.

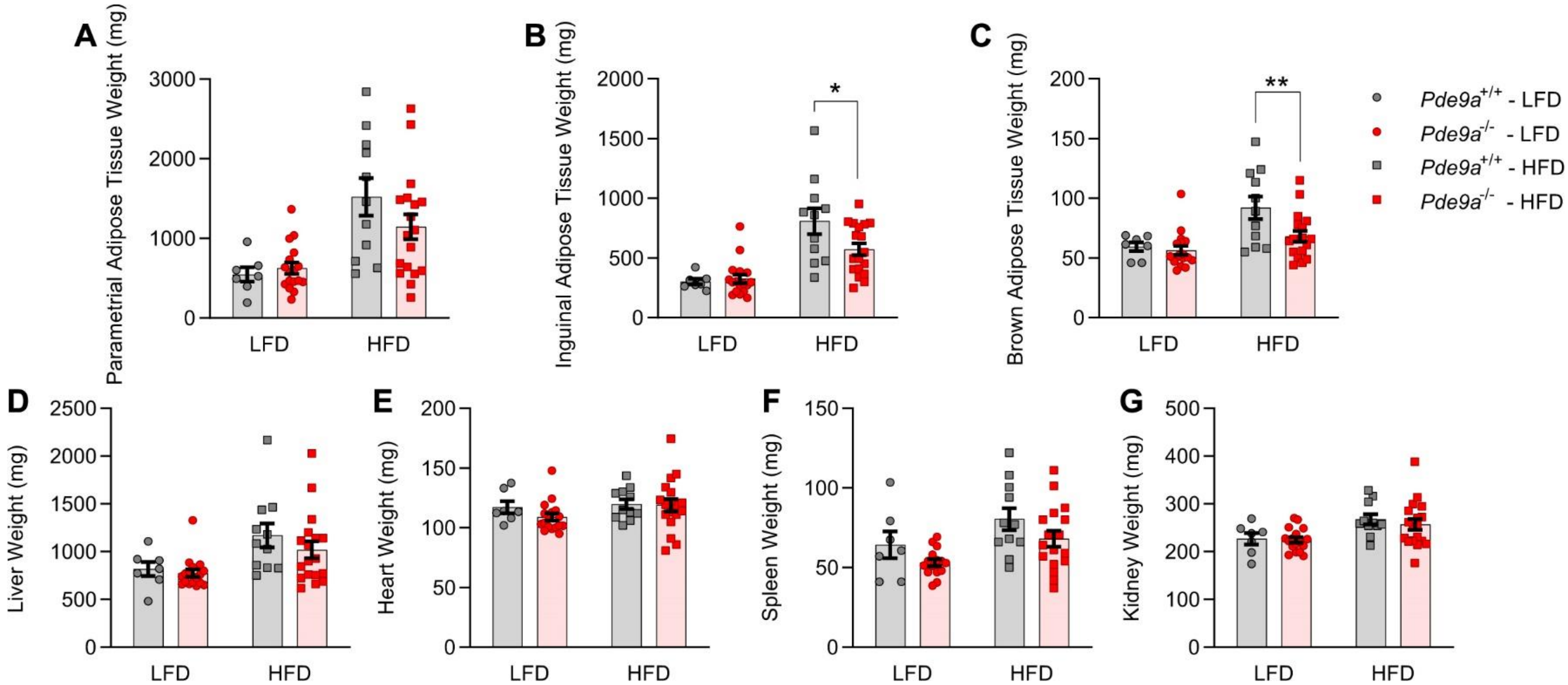


Supplemental Figure S3. Hyperinsulinemic euglycemic clamps revealed no differences in insulin sensitivity between weight matched *Pde9a*$^{+/+}$ and *Pde9a*$^{-/-}$ mice.

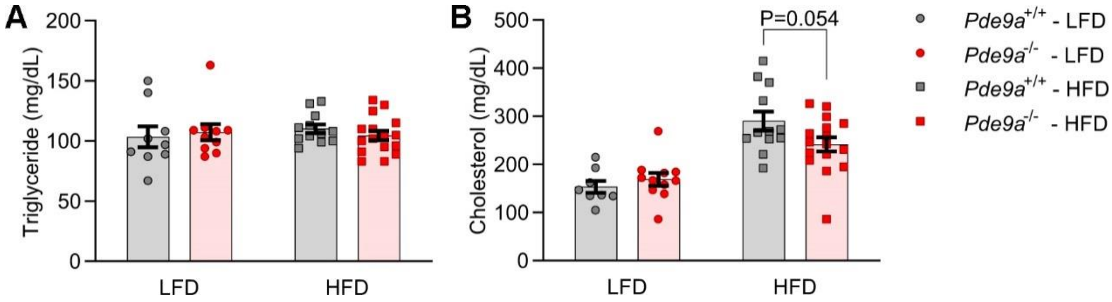
(A) IP-GTT in male *Pde9a*$^{+/+}$, *Pde9a*$^{+/-}$, and *Pde9a*$^{-/-}$ mice fed LFD for 15 weeks. (B) IP-ITT in male *Pde9a*$^{+/+}$, *Pde9a*$^{+/-}$, and *Pde9a*$^{-/-}$ mice fed LFD for 14 weeks. (C) Area under the curve (AUC) of ITT data in Figure 3C and Supplemental Figure 2B. (D) Glucose infusion rate. (E) Blood glucose during the clamp. (F) Tissue [^{14}C]2-deoxy-D-glucose uptake (Rg). (G) Fasting glucose turnover rate in *Pde9a*$^{-/-}$ mice compared to *Pde9a*$^{+/+}$ (P = 0.015). Glucose disappearance (Rd) and endogenous glucose production (Ra) during the clamp. Data are mean ± SEM. For (A) and (B), analyses was performed using 2-way ANOVA with repeated measures. Post-hoc analyses were performed using Sidak's multiple comparisons test. C and D were analyzed by multiple t-tests with statistical significance determined by the Holm-Sidak method with * P < 0.05. N = 5 *Pde9a*$^{+/+}$ HFD, 6 *Pde9a*$^{-/-}$ HFD.



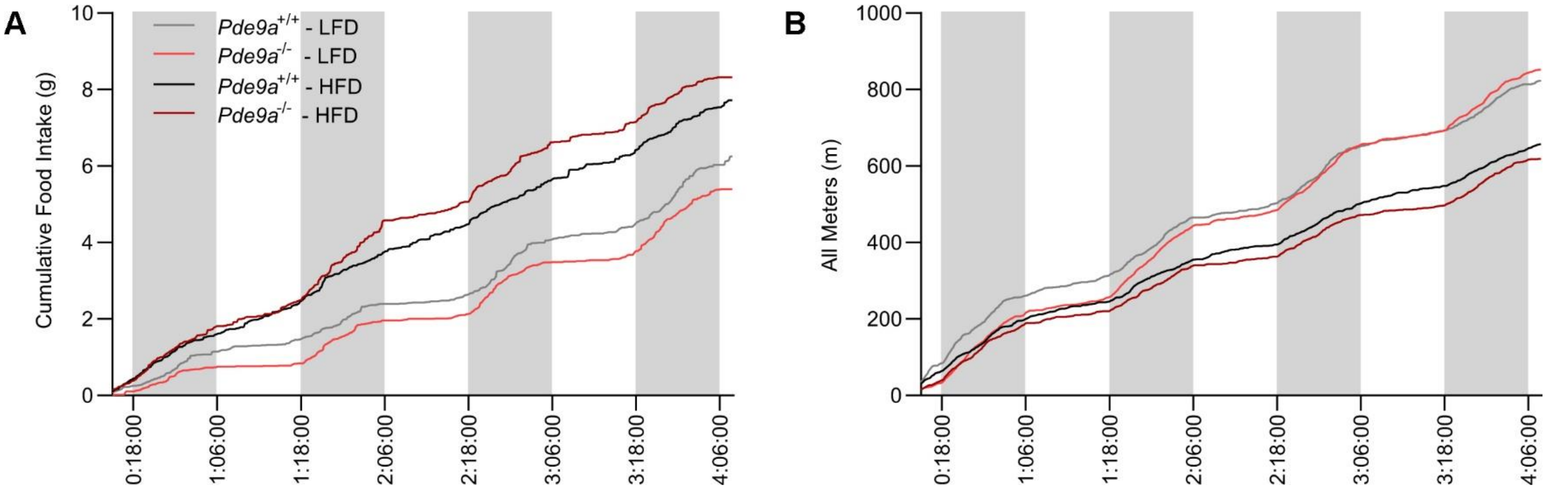
Supplemental Figure S4. Female *Pde9a*$^{-/-}$ mice have modest improvements in glucose homeostasis. IP-GTT in female *Pde9a*$^{+/+}$ and *Pde9a*$^{-/-}$ mice fed either HFD (A) (P = 0.022 effect of genotype) or LFD (B) for 15 weeks. (C) Area under the curve (AUC) of data in (A) and (B). IP-ITT in female *Pde9a*$^{+/+}$ and *Pde9a*$^{-/-}$ mice fed either HFD (D) (P = 0.059 effect of genotype) or LFD (E) for 14 weeks. (F) Area under the curve (AUC) of data in (D) and (E). Five-hour fasting (G) glucose and (H) insulin at the end of the study. Data are mean ± SEM. For (A, B, D, and E), 2-way ANOVAs were performed with repeated measures. For (C, G, and H), analyzed by 2-way ANOVA. (F) was analyzed by multiple t-tests. Post-hoc analyses were performed using Sidak's multiple comparisons test for *Pde9a* genotype only and are indicated on figures with * $P < 0.05$, ** $P < 0.01$ comparing *Pde9a*$^{+/+}$ vs. *Pde9a*$^{-/-}$. N = 7 *Pde9a*$^{+/+}$ LFD, 17 *Pde9a*$^{-/-}$ LFD, 11 *Pde9a*$^{+/+}$ HFD, 18 *Pde9a*$^{-/-}$ HFD.



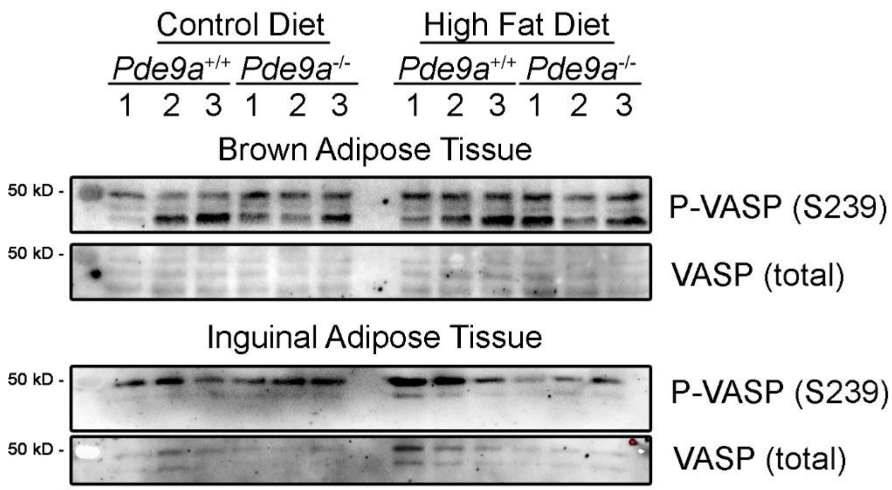
Supplemental Figure S5. ***Pde9a***$^{-/-}$ **female mice have reduce inguinal and brown adipose tissue weight.** (A) Parametrial WAT weights. (B) iWAT weights (P = 0.052, effect of genotype×diet interaction). (C) iBAT weights (P = 0.030, effect of genotype). (D) Liver weights. (E) Heart weights. (F) Spleen weights (P = 0.040, effect of genotype). (G) Kidney weights. Data are mean ± SEM. Analyses were performed using 2-way ANOVA. Post-hoc analyses were performed using Sidak's multiple comparisons test for *Pde9a* genotype only and are indicated on figures with * P < 0.05, ** P < 0.01 comparing *Pde9a*$^{+/+}$ vs. *Pde9a*$^{-/-}$. N = 7 *Pde9a*$^{+/+}$ LFD, 17 *Pde9a*$^{-/-}$ LFD, 11 *Pde9a*$^{+/+}$ HFD, 18 *Pde9a*$^{-/-}$ HFD.



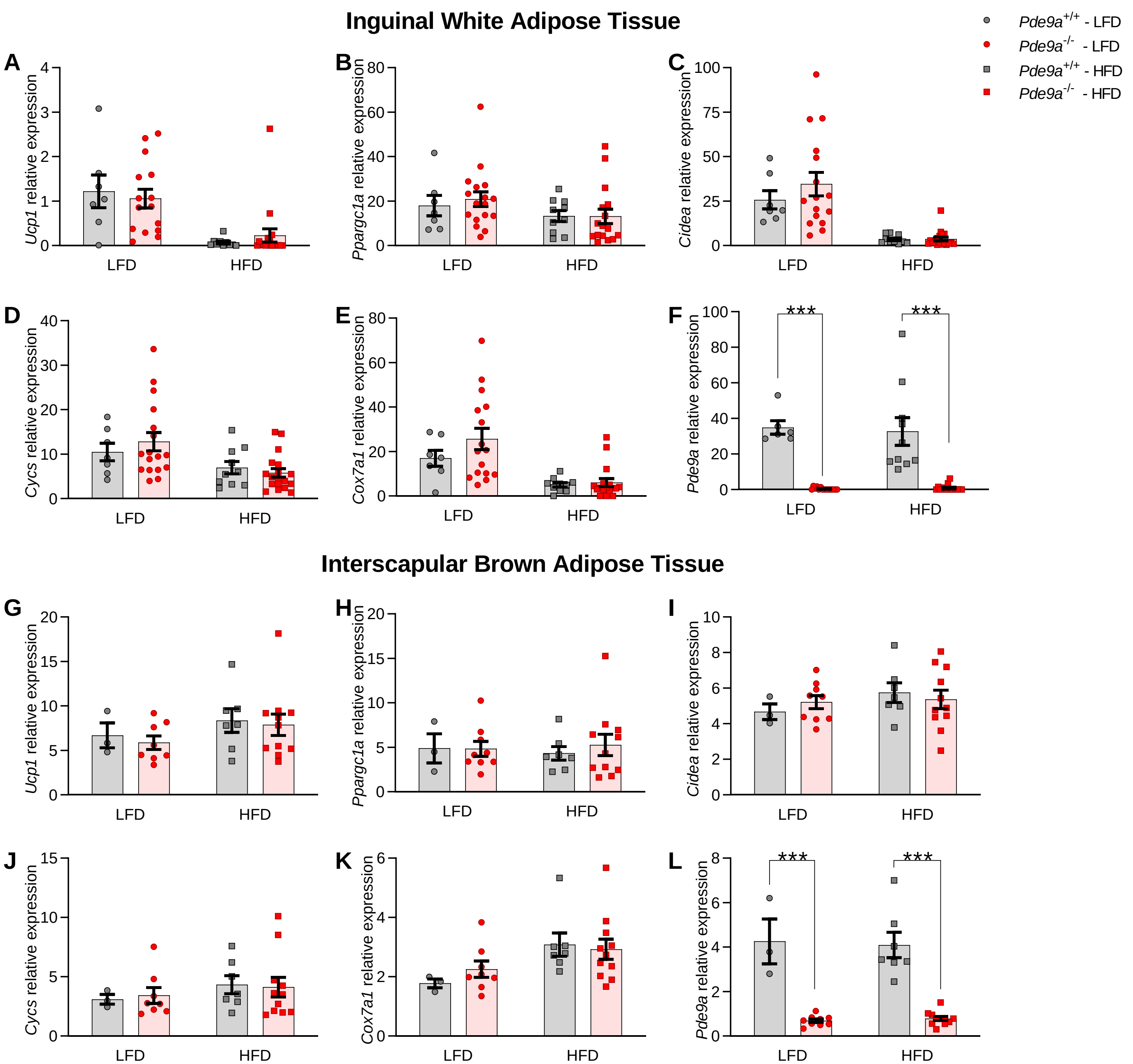
Supplemental Figure S6. Effect of PDE9 on circulating plasma lipids in *Pde9a*$^{+/+}$ and *Pde9a*$^{-/-}$ mice.
(A) Triglyceride concentrations in plasma from 5-hour fasted mice. (B) Cholesterol concentrations in plasma from 5-hour fasted mice. HFD fed *Pde9a*$^{+/+}$ *vs.* *Pde9a*$^{-/-}$ mice (P = 0.0621, effect of genotype×diet interaction). Data are mean ± SEM. Analyses were performed using 2-way ANOVA. Post-hoc analyses were performed using Sidak's multiple comparisons test for *Pde9a* genotype only and are indicated on figures. N = 9 *Pde9a*$^{+/+}$ LFD, 12 *Pde9a*$^{-/-}$ LFD, 11 *Pde9a*$^{+/+}$ HFD, 16 *Pde9a*$^{-/-}$ HFD.



Supplemental Figure S7. Food intake and physical activity.
(A) Cumulative food intake. (B) Cumulative fine and locomotor movement. Values in figure are the mean only from the Promethion System. N = 6 *Pde9a*$^{+/+}$ LFD, 7 *Pde9a*$^{-/-}$ LFD, 9 *Pde9a*$^{+/+}$ HFD, 8 *Pde9a*$^{-/-}$ HFD.



Supplemental Figure S8. VASP$^{(S239)}$ phosphorylation is not altered under basal conditions
P-VASP at Ser^{239} Western blot from male iBAT and iWAT.



Supplemental Figure S9. Female *Pde9a*$^{-/-}$ mice have unchanged adipose tissue thermogenic gene expression. The iWAT expression of (A) *Ucp1* (B) *Ppargc1a* (C) *Cidea* (D) *Cycs* (E) *Cox7a1* and (F) *Pde9a* and the iBAT expression of (G) *Ucp1* (H) *Ppargc1a* (I) *Cidea* (J) *Cycs* (K) *Cox7a1* and (L) *Pde9a* by qRT-PCR. Data are mean ± SEM. Analyses were performed using 2-way ANOVA. Post-hoc analyses were performed using Sidak's multiple comparisons test for *Pde9a* genotype only and are indicated on figures with *** $P < 0.001$ comparing *Pde9a*$^{+/+}$ vs. *Pde9a*$^{-/-}$. For iWAT, N = 6-7 *Pde9a*$^{+/+}$ LFD, 15-17 *Pde9a*$^{-/-}$ LFD, 10 *Pde9a*$^{+/+}$ HFD, 16-18 *Pde9a*$^{-/-}$ HFD. For iBAT, N = 3 *Pde9a*$^{+/+}$ LFD, 8-9 *Pde9a*$^{-/-}$ LFD, 7 *Pde9a*$^{+/+}$ HFD, 11 *Pde9a*$^{-/-}$ HFD.

Reagent	**Final Concentration**	**Source**	**Product Code**
Chemicals			
PF-04447943		MedChem Express	HY-15441
ANP (1-28)		AnaSpec Inc.	AS-20648
BNP		ProSpec	CYT-369-B
BAY 73-6691		Sigma	B3561
CL-316,243		American Cyanamid Co.	gift from Elliott Danforth Jr.
Isoproterenol		Sigma	I6504
pCPT-cGMP		Sigma	C5438
Kits			
Mouse Insulin ELISA		Mercodia	10-1247-01
Cell Culture			
IngJ6 and Bat8 Growth Medium			
DMEM/F12 GlutaMAX™		ThermoFisher	10565018
FBS	15%		
HEPES	2 mM		
penicillin and streptomycin	50 units/ml		
IngJ6 and Bat8 Differentiation Medium		days 1-4	
DMEM/F12 GlutaMAX™		ThermoFisher	10565018
FBS	10%		
dexamethasone	5 mM		
Insulin	0.5 mg/ml		
isobutylmethylxanthine (IBMX)	0.5 mM		
rosiglitazone	1 mM		
T3	1 nM		
IngJ6 and Bat8 Post-differentiation Maintenance Medium		days 5-8	
DMEM/F12 GlutaMAX™		ThermoFisher	10565018
FBS	10%		
Insulin	0.5 mg/ml		
T3	1 nM		
hMADS Growth Medium			
DMEM, low glucose		Lonza	12-707F
FBS	10%		
L-glutamine	2 mM		
HEPES	10 mM		

human FGF-2	2.5 ng/ml	Shenandoah Biotechnology	100-146
penicillin and streptomycin	50 units/ml		
hMADS Differentiation Medium		days 1-9	
DMEM/F12		Gibco	11039-021
Dexamethasone	1 µM		
IBMX	0.5 µM		
T3	0.2 nM		
insulin	5 µg/ml		
Rosiglitazone	1 µM		
transferrin	10 µg/ml		
hMADS Post-differentiation Maintenance Medium		days 10-12	
DMEM/F12		Gibco	11039-021
T3	0.2 nM		
insulin	5 µg/ml		
Rosiglitazone	1 µM		
transferrin	10 µg/ml		
hMADS Post-differentiation Basic Medium		days 13-16	
DMEM/F12		Gibco	11039-021
insulin	5 µg/ml		
transferrin	10 µg/ml		
Western Blotting Lysis Buffer			
HEPES	25 mM		
NaCl	150 mM		
EDTA	5 mM		
EGTA	5 mM		
glycerophosphate	5 mM		
Triton X-100	0.9%		
IGEPAL	0.1%		
sodium pyrophosphate	5 mM		
glycerol	10%		
cOmplete™ protease inhibitor cocktail	1 tablet/10 ml Lysis Buffer	Roche	4693124001
PhoSTOP phosphatase inhibitors	1 tablet/10 ml Lysis Buffer	Roche	4906845001
Antibodies			
AKT	1:1000	Cell Signaling Technology	9272

P-AKT(S473)	1:1000	Cell Signaling Technology	4060
VASP	1:1000	Cell Signaling Technology	3132
P-VASP(S293)	1:1000	Cell Signaling Technology	3114
β-actin	1:2000	Cell Signaling Technology	4967
UCP1 (Western Blot)	1:1000	Abcam	ab23841
UCP1 (IHC)		Abcam	ab10983
Goat Anti-Rabbit IgG –Alkaline Phosphatase	1:20000	MilliporeSigma	A3687
RNA Purification and Quantitative RT-PCR			
Trizol Reagent		Ambion	15596018
Zymo-Spin IIICG Column		Zymo Research	C1006-250-G
RNA Prep Buffer		Zymo Research	R1060-2-100
RNA Wash Buffer		Zymo Research	R1003-3-48
High-Capacity cDNA Reverse Transcription Kit		Applied Biosystems	4368814
PowerUp SYBR Green Master Mix		Applied Biosystems	A25742
GTT and ITT			
0.9% Saline		Hospira, Inc.	NDC 0409-7138-09
dextrose 50%		Agri Laboratories	NDC 57561-801-50
Insulin (Humulin R)		Lilly	HI-213
Oroboros Buffers and Reagents			
Malate 0.4 M			
Malic Acid	400 mM	Sigma	M1000
ph 7.0 with KOH			
Pyruvate 1 M			
Sodium Pyruvate	1 M	Sigma	P2256
ADP 0.5 M			
ADP	500 mM	Alfa Aesar	L14029
$MgCl_2 \cdot 6H_2O$	300 mM	Fisher	BP214
ph 7.0 with KOH			
Succinate 1M			
Succinic Acid	1 M	TCI	S0100
ph 7.0			

100 mM K_2EGTA buffer			
EGTA	100 mM	Sigma	E4378
KOH	200 mM	Fisher	P250
ph 7.0 with KOH			
100 mM CaK_2EGTA buffer			
$CaCO_3$	100 mM	Sigma	795445
EGTA	100 mM	Sigma	E4378
KOH	200 mM	Fisher	P250
ph 7.0 with KOH			
Biopsy Preservation Solution: BIOPS			
CaK_2EGTA buffer	2.77 mM		
K_2EGTA buffer	7.23 mM		
Na_2ATP	5.77 mM	Sigma	A2383
$MgCl_2$•$6H_2O$	6.56 mM	Fisher	BP214
Taurine	20 mM	Sigma	T0625
Na_2Phosphocreatine	15 mM	Sigma	P7936
Imidazole	20 mM	Sigma	56750
Dithiorthreitol	0.5 mM	Sigma	D9779
MES hydrate	50 mM	Sigma	M8250
Fatty Acid Free BSA	0.1%	Calbiochem	126575
ph 7.1 with KOH			
BIOPS+Saponin			
Saponin	50 ng/ml in BIOPS	Sigma	S7900
Mitochondrial Respiration Medium: MiR05			
EGTA	0.5 mM	Sigma	E4378
$MgCl_2$•$6H_2O$	3.0 mM	Fisher	BP214
Lactobionic acid	60 mM	RPI	L23000
Taurine	20 mM	Sigma	T0625
KH_2PO_4	10 mM	Fisher	P285
HEPES	20 mM	Sigma	H4034
Sucrose	110 mM	EMD	SX1075-1
Fatty Acid-Free BSA	0.1%	Calbiochem	126575
ph 7.1 with KOH			

Supplemental Table S1. List of Materials

Gene	**Forward**	**Reverse**	**Efficiency**
mRplp0	GATGCCCAGGGAAGACAG	ACAATGAAGCATTTTGGATAATCA	88.49% - 105.15%
mCidea	GTCTGCAAGCAACCAAAGAA	ATTGAGACAGCCGAGGAAGT	101.65% - 108.65%
mCox7a1	CGAAGAGGGGAGGTGACTC	AGCCTGGGAGACCCGTAG	101.2% - 108.29%
mCycs	ACCAAATCTCCACGGTCTGTTCGG	GGTGATGCCTTTGTTCTTGTTGGC	102.16% - 103.01%
mPde5a	ACAAAGGCATTGTGGGACAT	TTGGTCAACTTCTGCATTGAA	98.17% - 104.14%
mPde9a	AGATGGACATCTTGGTCCTGA	CGGGCATTGATCTGGTATGT	97.04% - 112.95%
mPpargc1a	CGGAAATCATATCCAACCAG	TGAGAACCGCTAGCAAGTTTG	97.02% - 107.41%
mUcp1	GGCCTCTACGACTCAGTCCA	TAAGCCGGCTGAGATCTTGT	93.57% - 111.9%

Supplemental Table S2. Primer Sequences

Fatty acid composition of hepatic triglycerides

Parameter	*Pde9a*$^{+/+}$ - LFD		*Pde9a*$^{-/-}$ - LFD		*Pde9a*$^{+/+}$ - HFD		*Pde9a*$^{-/-}$ - HFD		ANOVA p-value		
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	Genotype	Diet	Interaction
	g/100 g fatty acids										
12:0	0.38	0.11	0.28	0.08	2.05	0.37	2.12	0.24	0.974	<0.001	0.736
14:0	1.74	0.17	1.60	0.11	5.05	0.36	5.77	0.38	0.366	<0.001	0.193
16:0	25.49	0.76	27.28	0.38	31.31	0.54	31.22	0.51	0.137	<0.001	0.101
16:1	8.80	0.31	8.07	0.46	7.63	0.29	7.06	0.35	0.081	0.005	0.825
17:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA	NA
18:0	1.98	0.23	2.04	0.22	2.64	0.20	2.76	0.19	0.678	0.002	0.895
18:1ω9	42.00	0.87	42.08	0.78	36.60	0.88	35.77	0.66	0.642	<0.001	0.574
18:1ω7	7.29	0.50	7.59	0.42	5.56	0.47	5.42	0.25	0.848	<0.001	0.596
18:2	10.25	0.74	9.72	0.64	6.63	0.57	7.15	0.54	0.989	<0.001	0.404
18:3ω6	0.02	0.02	0.00	0.00	0.06	0.03	0.11	0.04	0.657	0.016	0.210
18:3ω3	0.49	0.04	0.30	0.06	0.27	0.04	0.25	0.07	0.083	0.038	0.184
20:3ω6	0.31*	0.06	0.12*	0.05	0.27	0.03	0.25	0.04	0.029	0.334	0.080
20:4	0.66	0.08	0.50	0.11	0.71	0.11	0.79	0.09	0.716	0.107	0.237
20:5	0.00	0.00	0.00	0.00	0.04	0.02	0.09	0.05	0.412	0.033	0.412
22:4ω6	0.04	0.03	0.06	0.03	0.19	0.04	0.18	0.03	0.904	<0.001	0.629
22:5ω6	0.04	0.03	0.04	0.03	0.19	0.03	0.21	0.04	0.769	<0.001	0.867
22:5ω3	0.01	0.01	0.04	0.03	0.29	0.06	0.25	0.04	0.935	<0.001	0.414
22:6	0.50	0.07	0.27	0.08	0.51	0.11	0.60	0.08	0.472	0.065	0.088
	Ratio										
16:0/16:1	2.93	0.13	3.50	0.22	4.16	0.14	4.56	0.20	0.013	<0.001	0.634
18:0/18:1	0.04	0.005	0.04	0.005	0.06	0.006	0.07	0.006	0.654	<0.001	0.810
Saturated / Unsaturated†	0.42	0.02	0.45	0.01	0.70	0.02	0.72	0.02	0.142	<0.001	0.813

†(12:0+14:0+15:0+16:0+17:0+18:0) / (16:1+18:1ω9+18:1ω7+18:2+18:3ω6+18:3ω3+20:3ω6+20:4+20:5+22:4ω6+22:5ω6+22:5ω3+22:6)

* Sidak post-hoc comparison P<0.05

** Sidak post-hoc comparison P<0.01

*** Sidak post-hoc comparison P<0.001

Supplemental Table S3. Fatty acid composition of hepatic triglycerides.

Fatty acid composition of hepatic triglycerides from male *Pde9a*$^{+/+}$ and *Pde9a*$^{-/-}$, fed either LFD or HFD. N = 10 *Pde9a*$^{+/+}$ LFD, 11 *Pde9a*$^{-/-}$ LFD, 13 *Pde9a*$^{+/+}$ HFD, 16 *Pde9a*$^{-/-}$ HFD.