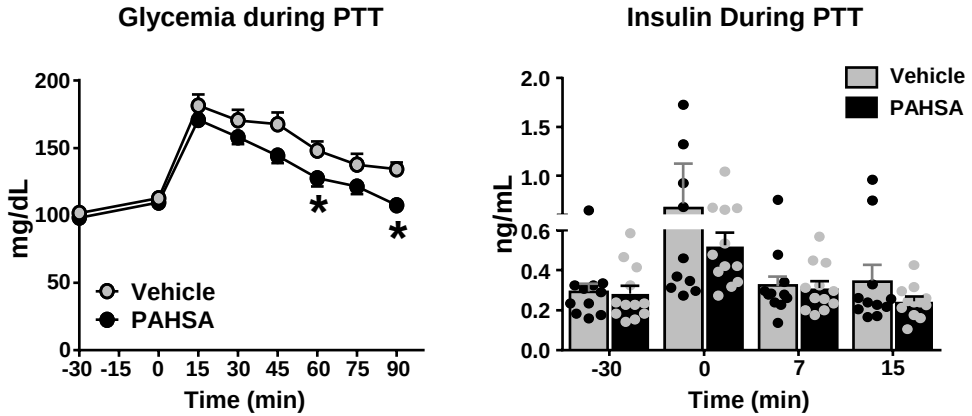
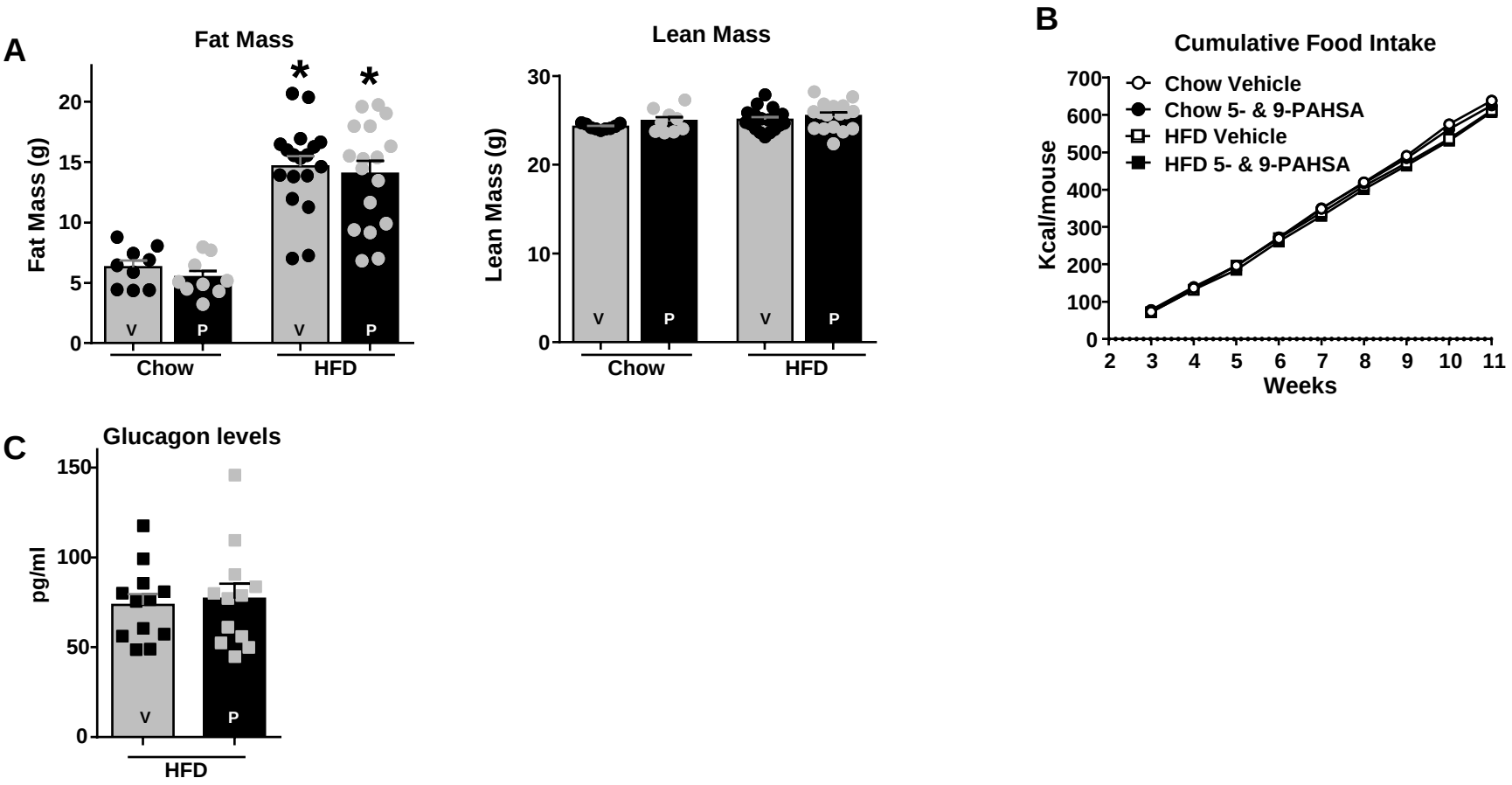
Fig. S1



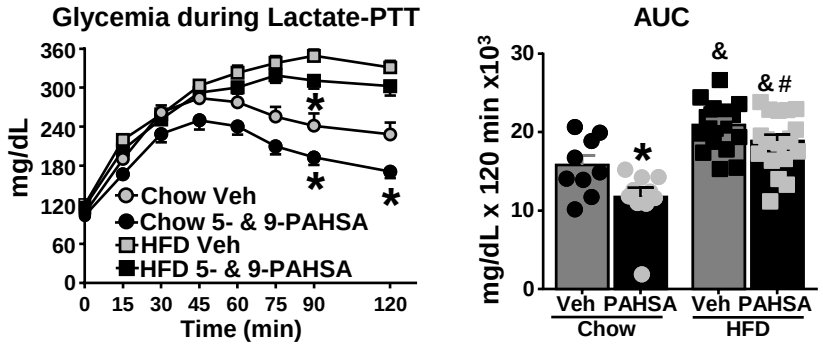
Supplemental Figure 1: A single oral dose of 5- and 9-PAHSAs decreases glycemia during pyruvate tolerance test (PTT) in chow-fed mice without changing insulin levels compared to vehicle-treated mice. Glucose (left) and Insulin (right) levels during a pyruvate tolerance test (PTT). 17.5 hr after food removal, chow-fed mice were gavaged with 5- and 9-PAHSA (30mg/kg BW, 50% of each) (at -30 min) or an equivalent volume of vehicle (50%PEG-400, 0.5% Tween-80, 49.5% distilled water). Thirty min later (at 0 min), an intraperitoneal PTT (2g/kg BW) was performed. Serum glucose and insulin levels were measured during pyruvate challenge. n=10-12 mice/group. *$p<0.05$ vs vehicle. Data are presented as means ± SEM. Statistical significance was evaluated by two-way ANOVA with Tukey post-hoc tests.

Fig. S2



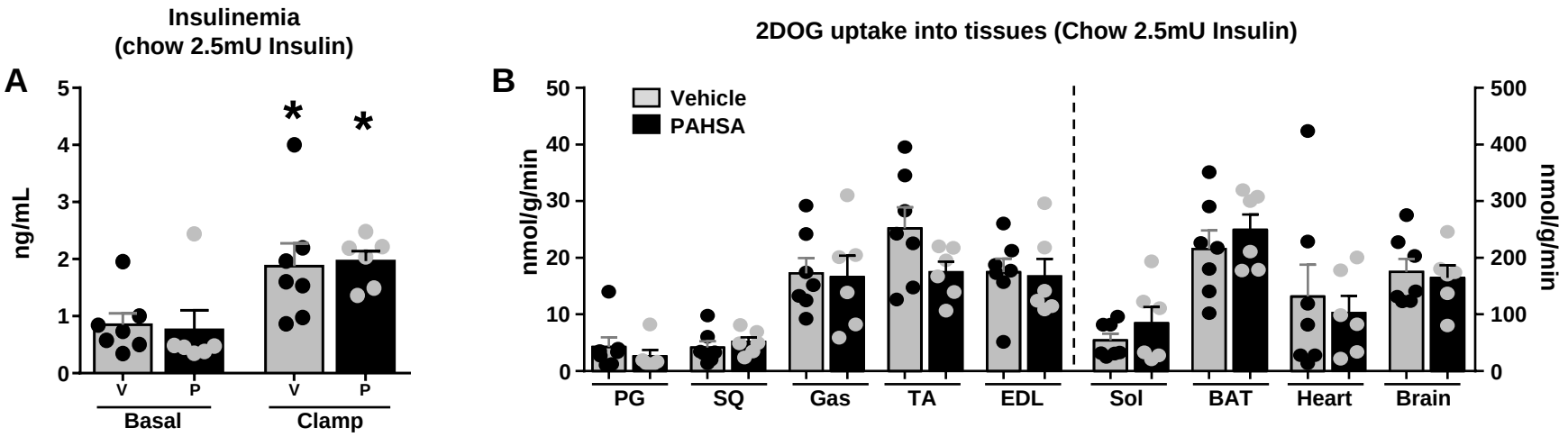
Supplemental Figure 2: Effects of chronic PAHSA treatment on fat mass, lean mass, food intake in chow- and HFD-fed mice and glucagon levels in HFD-fed mice. (A) Fat mass (left panel), lean mass (right panel) and (B) food intake were measured in mice treated with 5-PAHSA (0.1 mg/day) and 9-PAHSA (0.4 mg/day) or vehicle (50%PEG-400, 0.5% Tween-80, 49.5% distilled water) delivered by subcutaneous osmotic minipumps coinciding with the initiation of chow or HFD feeding for 13 weeks. Chow: n=9, HFD: n=17-18. *p<0.05 vs chow. (C) Glucagon levels in mice treated with vehicle or 5- and 9-PAHSA for 5 weeks by subcutaneous minipumps. Serum was collected after 5hr food removal in tubes containing Aprotinin (25KIU/μL). n=12. Data are presented as means ± SEM. Statistical significance was evaluated by two-way ANOVA with Tukey post-hoc tests or unpaired two-tailed student's t-test. V=vehicle-treated; P=PAHSA-treated.

Fig. S3



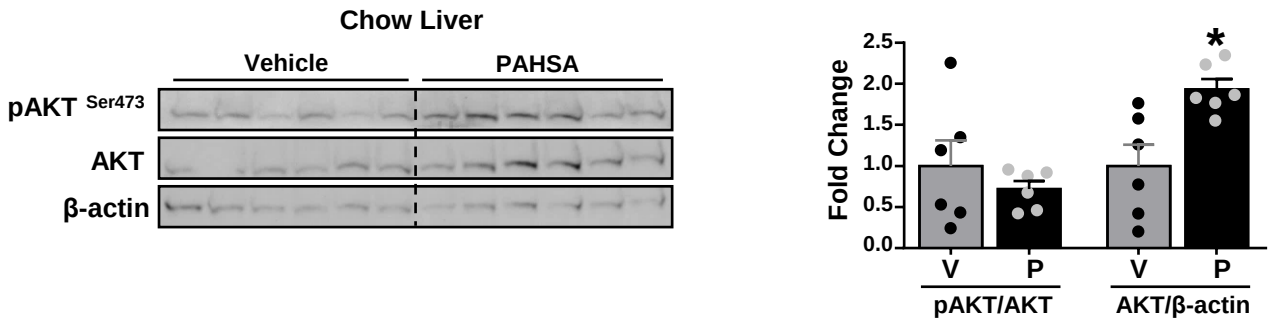
Supplemental Figure 3: Chronic PAHSA treatment decreases glycemia from pyruvate and lactate in chow- and HFD-fed mice. 18hr after food removal, mice were intraperitoneally injected with pyruvate and lactate (1:10, 2g/kg BW) after 4 weeks of treatment with either vehicle (50%PEG-400, 0.5% Tween-80, 49.5% distilled water) or 5- and 9-PAHSA by subcutaneous minipumps. Chow: n=9-10, HFD: n=17-19. Incremental area under the curve (AUC) was calculated from 0 to 120 min. *$p<0.05$ versus vehicle within same diet, $^{\&}p<0.05$ versus vehicle-treated mice on chow diet, $^{\#}p=0.06$ versus vehicle-treated mice on HFD. Data are presented as means ± SEM. Statistical significance was evaluated by two-way ANOVA with Tukey post-hoc tests. Veh=vehicle, PAHSA=5- and 9-PAHSA.

Fig. S4



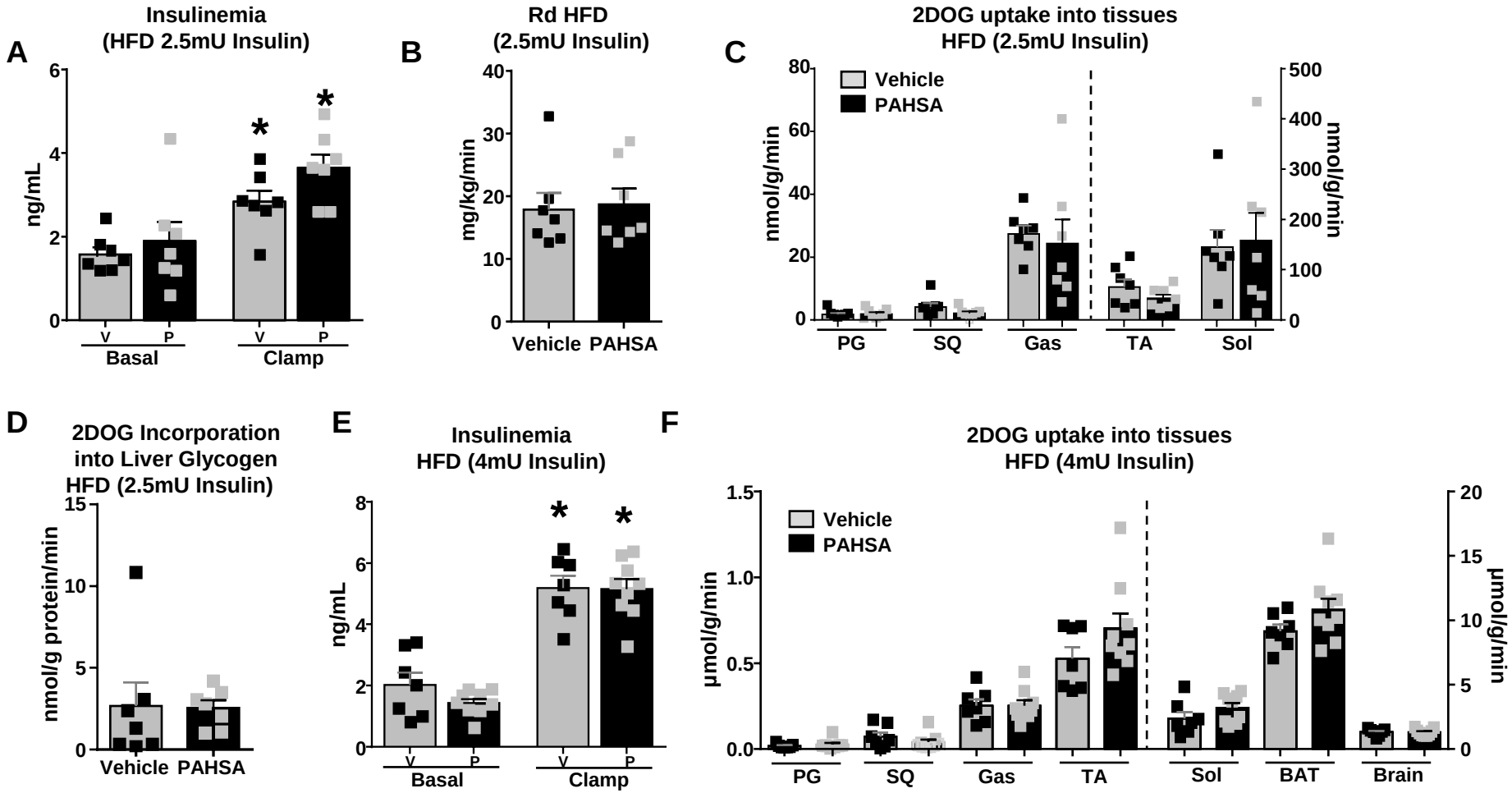
Supplemental Figure 4: Effect of chronic PAHSA treatment on 2-DOG uptake into tissues during clamp in mice on chow diet. (A) Serum insulin levels during hyperinsulinemic euglycemic clamp (2.5 mU/min/kg insulin infusion rate) in chow-fed mice. B) *In vivo* 2-DOG uptake into tissues in chow-fed mice at the end of the 2.5 mU insulin clamp. 2DOG uptake was measured by the ion-exchange column method. n=6-7/group. *p<0.05 vs basal. Data are presented as means ± SEM. Statistical significance was evaluated by two-way ANOVA with Tukey post-hoc tests. V=vehicle-treated; P=PAHSA-treated; PG= perigonadal white adipose tissue; SQ= subcutaneous white adipose tissue; Gas=gastrocnemius; TA=tibialis anterior; EDL=Extensor digitorum longus muscle; Sol= soleus; BAT= brown adipose tissue.

Fig. S5



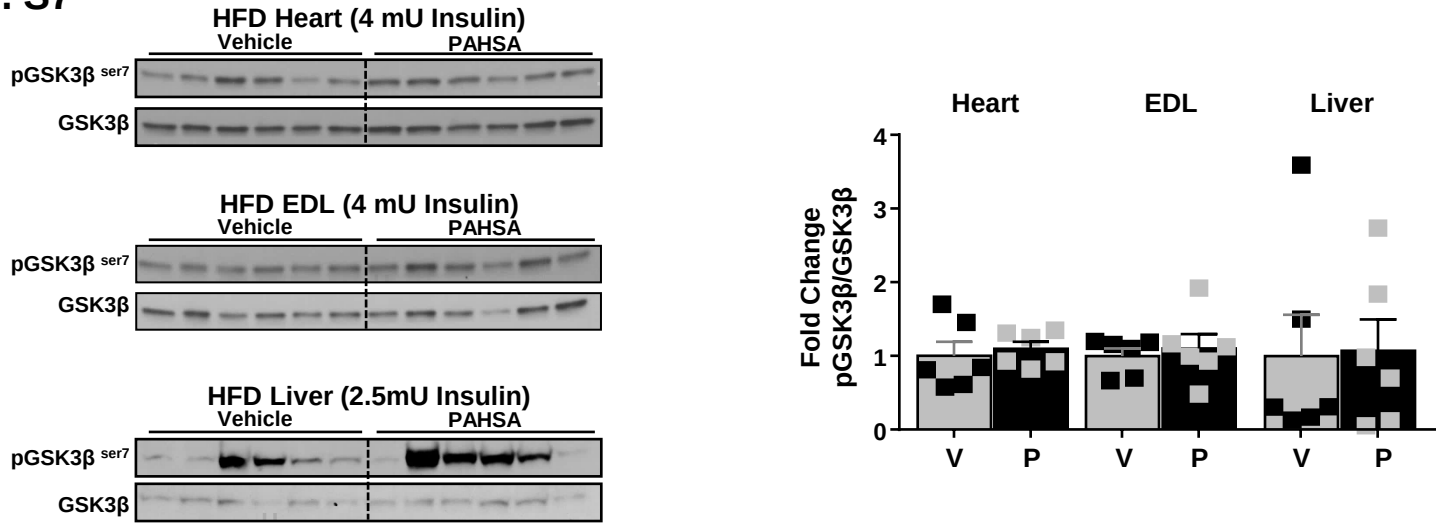
Supplemental Figure 5: PAHSA treatment increases the phosphorylation and the total amount of Akt in mice on chow diet. Immunoblotting (Left) and densitometry quantification (Right) of hepatic AKT phosphorylation and total AKT, and beta-actin at the end of the 2.5 mU (insulin infusion rate) clamp. n=6/group. *p<0.05 vs vehicle. Data are presented as means ± SEM. Statistical significance was evaluated by t-test. V=vehicle-treated; P=PAHSA-treated.

Fig. S6



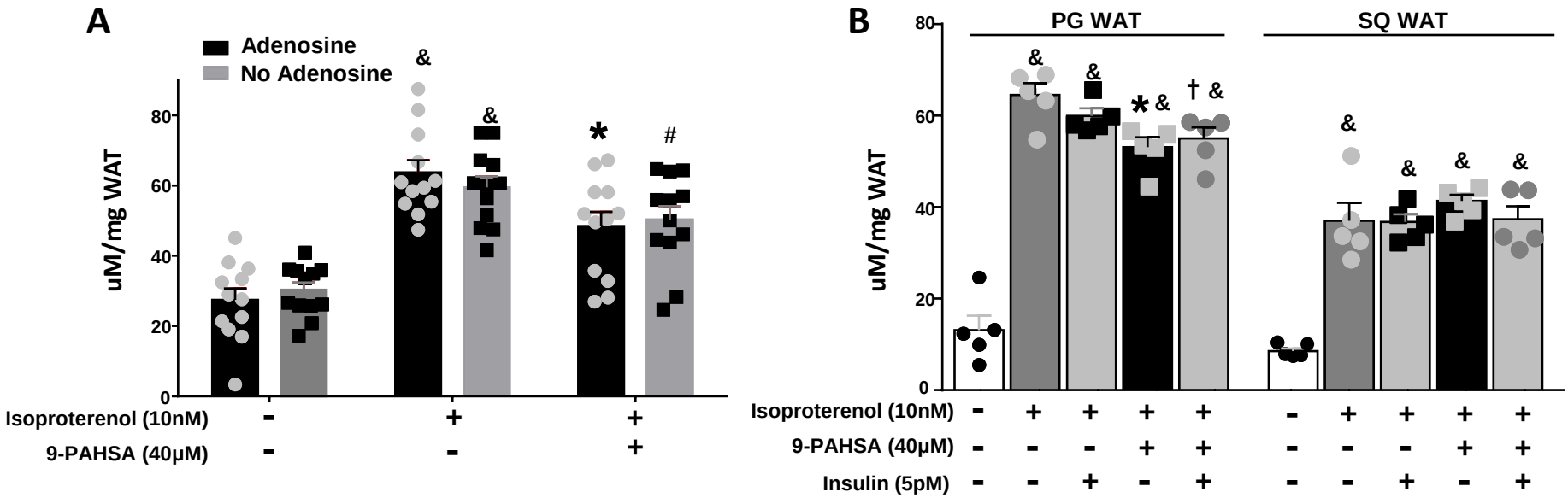
Supplemental Figure 6: Effects of chronic PAHSA treatment on 2-DOG uptake into tissues in HFD-fed mice. (A-F) Hyperinsulinemic euglycemic clamp studies were performed in HFD mice treated with vehicle or 5- and 9-PAHSA in combination for 13 weeks. Insulin levels (A), Rate of disappearance of glucose (Rd) (B), *in vivo* 2-DOG uptake (C) and 2-DOG incorporation into liver glycogen (D) during clamp performed with 2.5 mU/min/kg insulin infusion rate. Insulin levels (E) and *in vivo* 2-DOG uptake (F) during clamp using 4 mU/min/kg insulin infusion rate. 2-DOG uptake was measured by the perchloric acid method. n = 7–9/group. *p<0.05 vs basal. Data are presented as means ± SEM. Statistical significance was evaluated by two-way ANOVA with Tukey post-hoc tests, or unpaired two-tailed student's t-test. V=vehicle-treated; P=PAHSA-treated; PG= perigonadal white adipose tissue; SQ= subcutaneous white adipose tissue; Gas=gastrocnemius; TA=tibialis anterior; Sol= soleus; BAT= brown adipose tissue.

Fig. S7



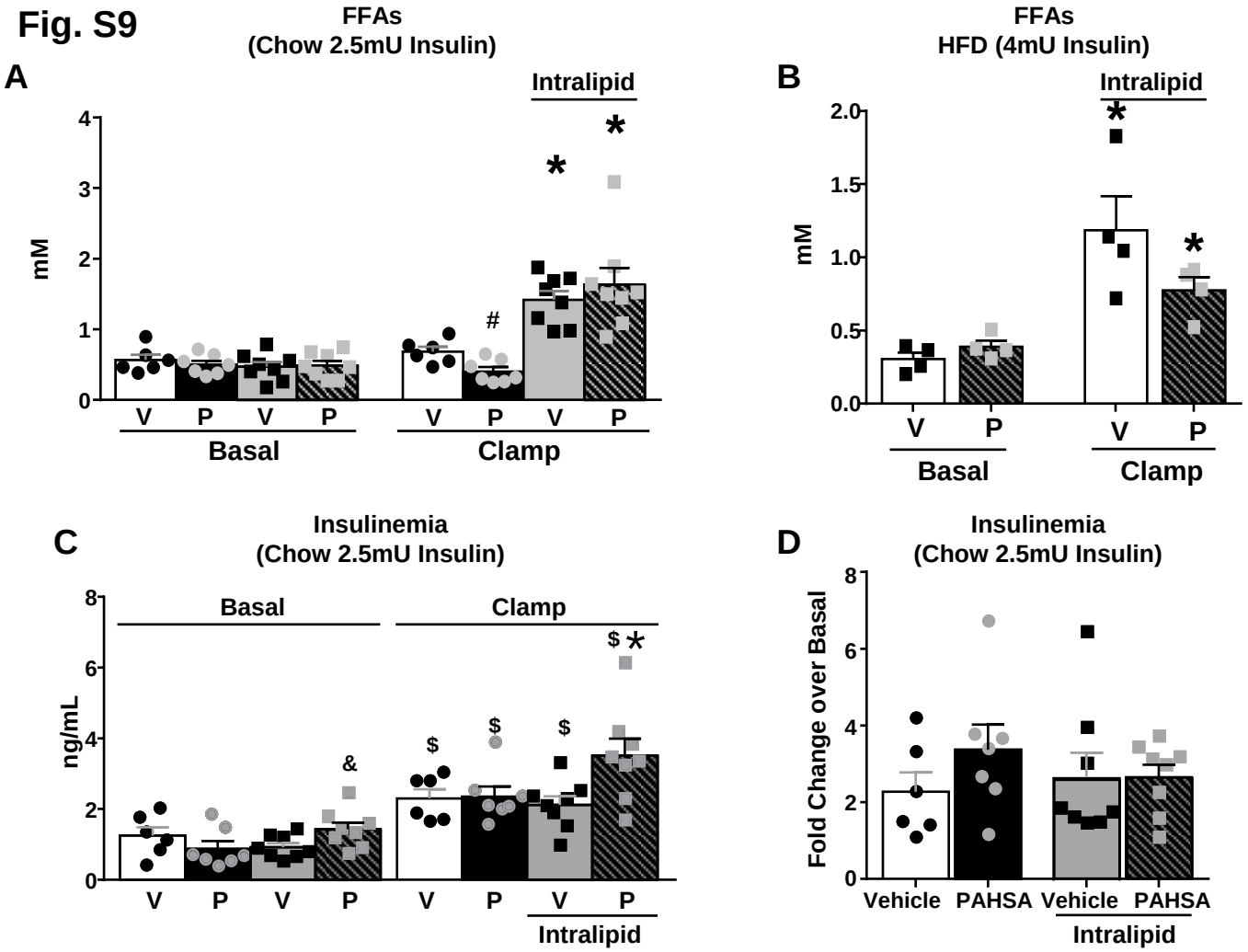
Supplemental Figure 7: Effects of chronic PAHSA treatment on phosphorylation and total amount of GSK3β in mice on HFD diet. Immunoblotting (Left) and densitometry quantification (Right) of GSK3β phosphorylation and total protein in heart, extensor digitorum longus muscle (EDL) and liver at the end of the 2.5 mU or 4 mU clamp. n=6/group. Data are presented as means ± SEM. Statistical significance was evaluated by t-test. V=vehicle-treated; P=PAHSA-treated.

Fig. S8



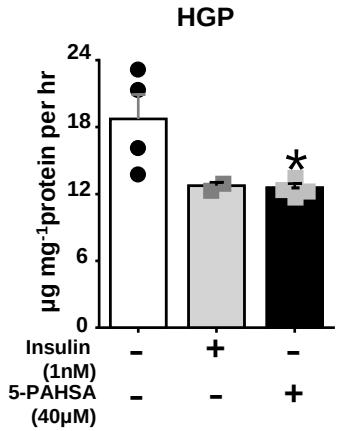
Supplemental Figure 8: Effect of 9-PAHSA on lipolysis in mouse WAT explants. A) Adenosine does not alter the effect of 9-PAHSA to decrease lipolysis. FFA release from perigonadal (PG) WAT explants with or without adenosine. n=12/group. B) The effect of 9-PAHSA to inhibit lipolysis is not additive with insulin at a submaximal insulin concentration. Free fatty acid release from PG and subcutaneous (SQ) WAT explants. n=5/group. $^{\&}p<0.05$ versus untreated cells, *$p<0.05$ versus cells treated only with isoproterenol (ANOVA), $^{\#}p=0.07$ versus cells treated only with isoproterenol (t-test), $^{\dagger}p<0.05$ versus cells treated only with isoproterenol (t-test). Data are presented as means ± SEM. Statistical significance was evaluated by two-way ANOVA with Tukey post-hoc tests and student's t-test.

Fig. S9



Supplemental Figure 9: Serum Free Fatty Acid (FFA) levels during Intralipid infusion in chronic PAHSA-treated mice. (A) Serum FFA levels during clamp (2.5 mU/min/kg insulin infusion rate) in chow-fed mice. n=6-8/group. $^{\#}p<0.05$ vs vehicle without Intralipid infusion at the end of clamp (Clamp), *p<0.05 vs basal and no Intralipid infusion during clamp for both Vehicle and PAHSA. (B) Serum FFA levels during clamp (4 mU/min/kg insulin infusion rate) in HFD-fed mice. n=4/group. *p<0.05 vs basal. (C) Serum insulin levels during clamp (2.5 mU/min/kg insulin infusion rate) in chow-fed mice. n=6-8/group. $^{\&}p<0.05$ vs the vehicle group in the basal state that will receive intralipid (gray bar), $^{\$}p<0.05$ vs basal groups, *p<0.05 vs vehicle group with Intralipid infusion during clamp. (D) Insulin fold change over basal condition (2.5 mU/min/kg insulin infusion rate). n=6-8/group. Data are means ± SEM. Statistical significance was evaluated by two-way ANOVA with Tukey post-hoc tests. V=vehicle-treated; P=PAHSA-treated.

Fig. S10



Supplemental Figure 10: Effects of 5-PAHSA on hepatic glucose production in murine primary hepatocytes. (A) Mouse primary hepatocytes were treated with DMSO (0.2%, white bar), insulin or 5-PAHSA. 3hr after treatment, glucose output was measured. n=2-4 wells/condition. *p<0.05 versus DMSO control cells (white bar). Data are means ± SEM. Statistical significance was evaluated by one-way ANOVA with Tukey post-hoc tests. HGP=hepatic glucose production.

Table S1

Glycemia and Glucose infusion rate (GIR) during the last 40 min of the hyperinsulinemic-euglycemic clamps with concurrent Intralipid infusion						
Experimental group	**Mice on chow diet**				**Mice on HFD**	
Solution infused during the clamp	**Saline+heparin**		**Intralipid+heparin**		**Intralipid+heparin**	
Treatment	**VEHICLE**	**PAHSA**	**VEHICLE**	**PAHSA**	**VEHICLE**	**PAHSA**
Number of mice	6	7	8	8	4	4
Glycemia (mg/dL) Data are means ± SE of five measurements per mouse during the last 40 min of the clamp period.	121 ± 5	119 ± 3	111 ± 3	114 ± 2	105 ± 8	118 ± 3
	116 ± 2	107 ± 8	103 ± 5	121 ± 3	102 ± 6	112 ± 3
	117 ± 2	115 ± 4	119 ± 5	104 ± 2	134 ± 2	114 ± 1
	120 ± 5	117 ± 2	118 ± 4	112 ± 3	122 ± 2	123 ± 2
	123 ± 4	111 ± 4	111 ± 2	109 ± 7		
	125 ± 6	105 ± 6	117 ± 7	106 ± 5		
		117 ± 4	123 ± 5	125 ± 9		
			111 ± 4	130 ± 3		
Glucose Infusion Rate (GIR, mg/Kg/min) Data are means ± SE of five measurements per mouse during the last 40 min of the clamp period.	22 ± 1	40 ± 0	9 ± 1	21 ± 1	12 ± 1	14 ± 0
	3 ± 0	10 ± 1	28 ± 1	20 ± 1	17 ± 1	10 ± 0
	7 ± 0	37 ± 1	14 ± 0	17 ± 0	1 ± 0	17 ± 1
	9 ± 0	3 ± 0	12 ± 1	18 ± 1	12 ± 0	16 ± 1
	6 ± 1	25 ± 0	10 ± 0	18 ± 2		
	8 ± 1	31 ± 2	5 ± 1	16 ± 1		
		25 ± 1	4 ± 0	1 ± 0		
			11 ± 0	0 ± 0		