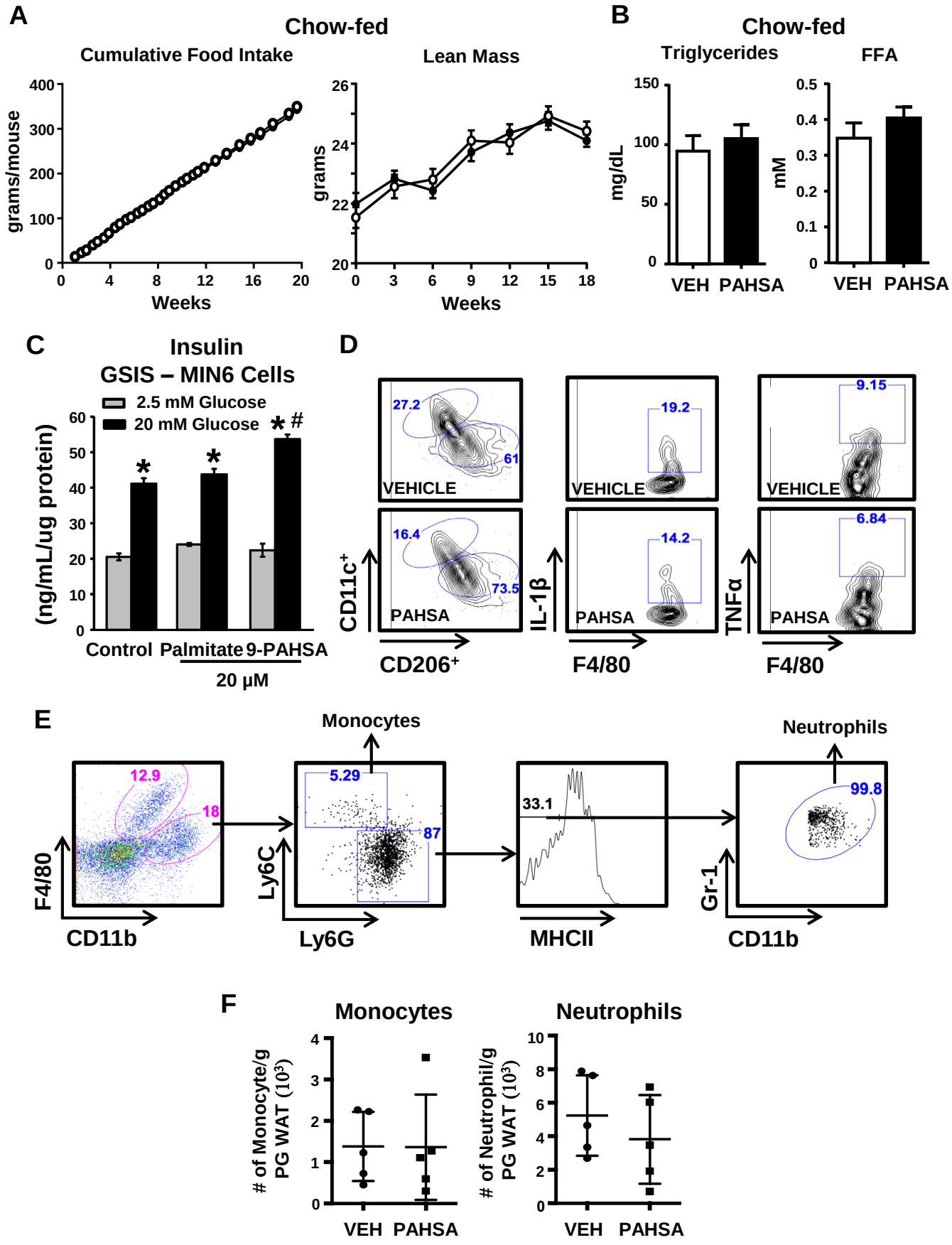
Figure S1. Related to Figure 1.



<u>Figure S1.</u> Chronic PAHSA treatment in chow-fed mice does not affect food intake, lean mass, serum triglycerides, free fatty acids or the number of PG WAT monocytes and neutrophils. Related to Figure 1.

(A) Food intake and lean mass were measured in chow-fed mice treated with vehicle or 5- and 9-PAHSA- (2 mg/kg body weight/day of each) delivered by subcutaneous minipumps for 5 months. n=16/group. (B) Serum triglycerides and free fatty acids (FFA) were measured after 5 months of treatment. Data are means±SEM. (C) Insulin secretion from MIN6 cells treated with DMSO, 9-PAHSA (20 μM), or palmitate (20 μM) for 45 minutes during glucose (20 mM) stimulation. *$p<0.05$ vs. respective 2.5mM glucose, #$p<0.05$ vs. Control 20mM glucose and palmitate. (D) Gating strategies for measuring the number of CD11c+and CD206+macrophages, and macrophages expressing IL-1β and TNF-α in PG WAT of vehicle- and PAHSA-treated mice after 5 months of treatment. (E) Gating strategies for measuring PG WAT monocytes and neutrophils from same mice as in (D). (F) The total number of PG WAT monocytes and neutrophils from vehicle and PAHSA-treated mice. n=4-5/group. Statistical significance was evaluated by unpaired two-tailed Student's t-test. Data are means±SEM.

Figure S2. Related to Figure 2.

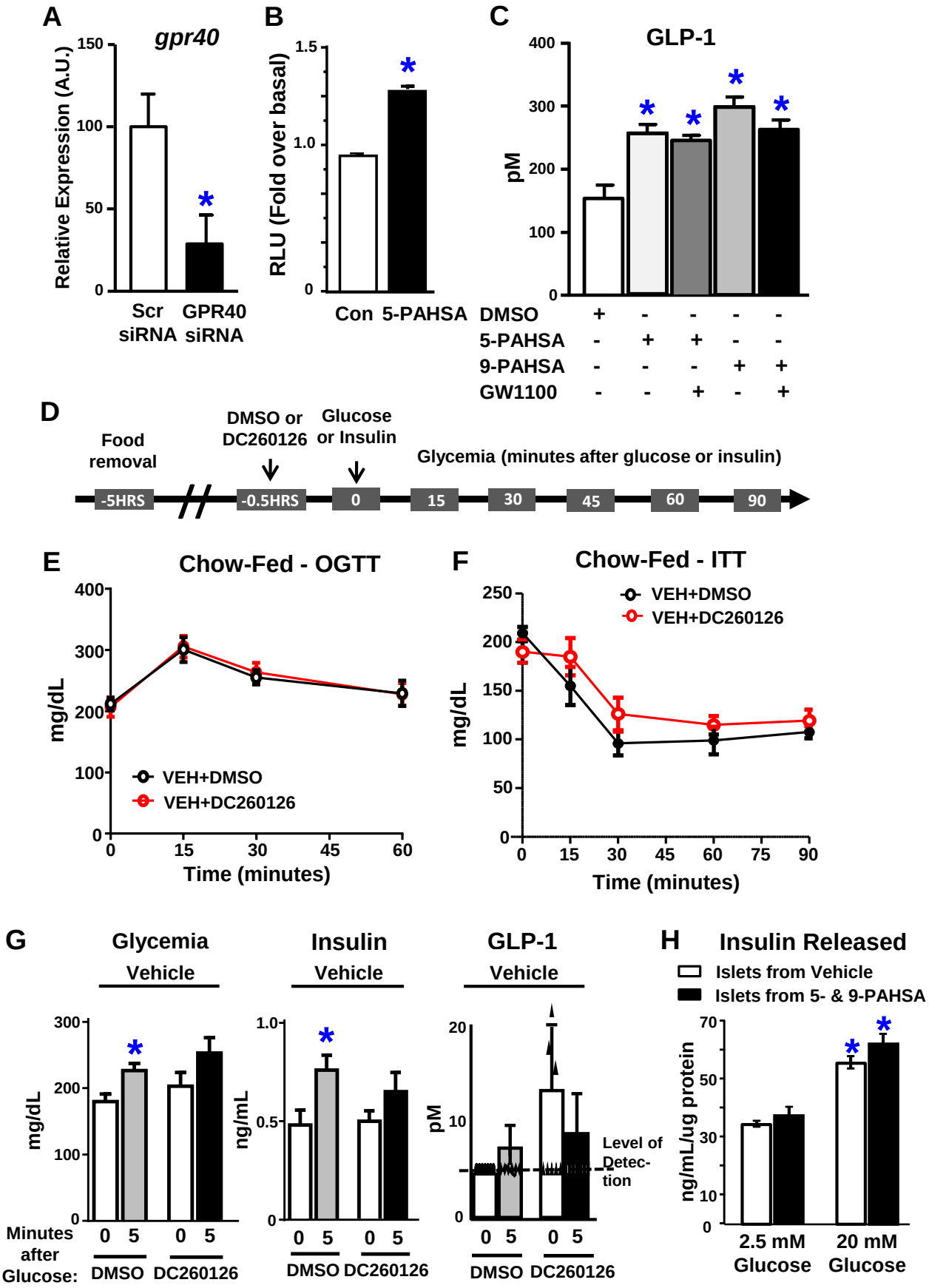
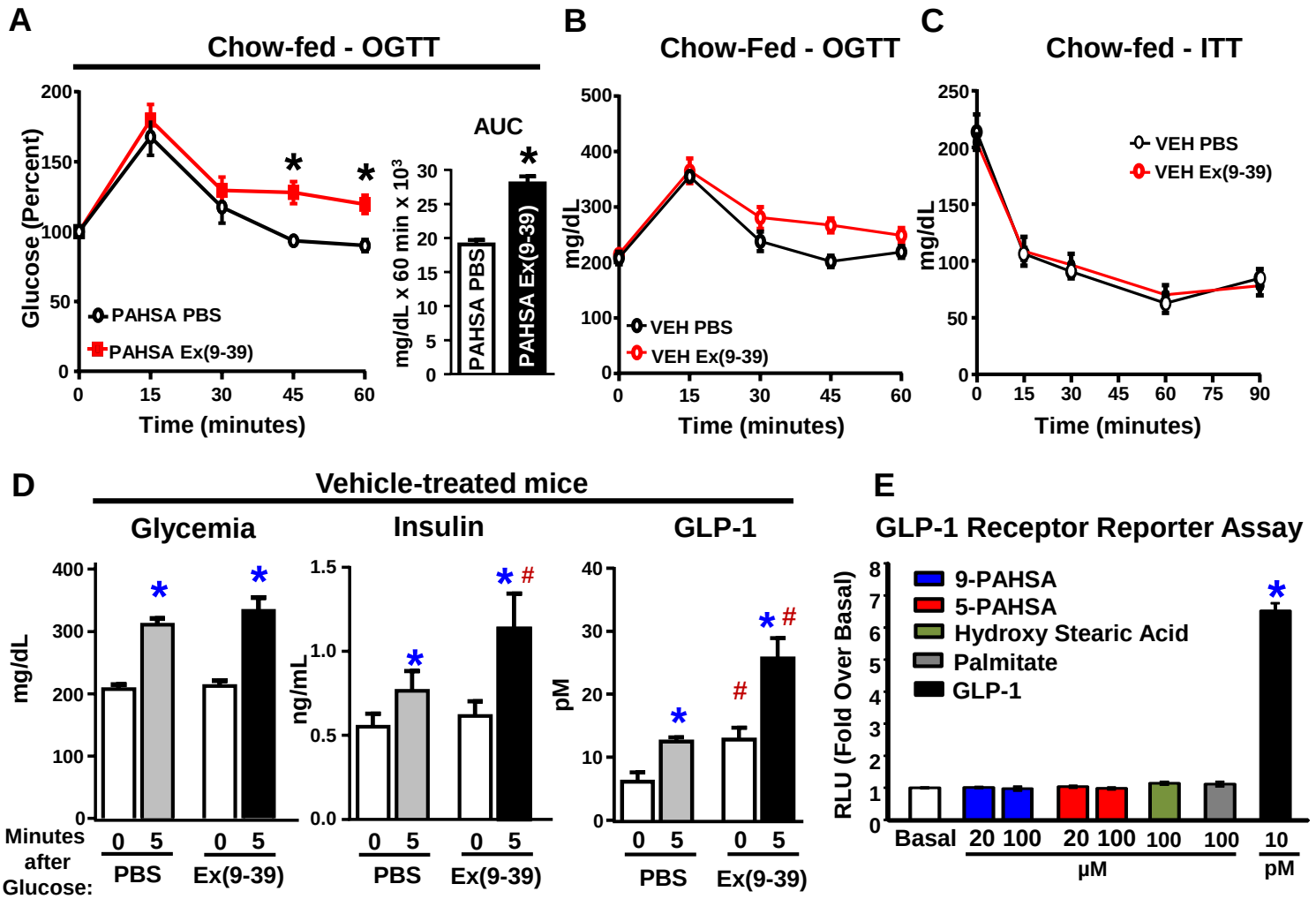


Figure S2: Effects of GPR40 antagonism on PAHSA activation of GPR40, PAHSA stimulation of GLP-1 secretion and on glucose-insulin homeostasis in vehicle-treated mice. Related to Figure 2.

(A) GRP40 mRNA expression in MIN6 cells transfected with scrambled or GPR40 siRNA. n=6 wells/condition. *p<0.05 vs. scrambled siRNA. (B) GPR40 reporter assay in HEK293 cells treated with control or 20 μM 5-PAHSA. n=3 wells/condition. (C) Total GLP-1 secretion in STC-1 cells pre-treated with 5-PAHSA or 9-PAHSA (20 μM) with or without GW1100 (10 μM) followed by acute stimulation with glucose. n=6 wells/condition, *p<0.05 vs. DMSO. (D) 5-hours after food removal vehicle-treated mice were injected with either DMSO or DC260126 (5 mg/kg body weight dose) intraperitoneally followed by a glucose gavage or insulin I.P. injection after 30 minutes for an OGTT (E) or ITT (F). n=14-16/group. *p<0.05 vs. vehicle DMSO. (G) Following the same protocol as (D), glycemia, insulin and GLP-1 levels were measured in vehicle-treated mice at baseline and 5 minutes after a glucose gavage. Most values for GLP-1 secretion were below the assay detection limit. n=14-16/group. *p<0.05 vs. baseline within same treatment. Data are means±SEM. (H) Insulin secretion in islets isolated from vehicle and PAHSA-treated mice after 5 months of treatment and stimulated with either 2.5 or 20 mM glucose. n=7 mice/group. *p<0.05 vs. 2.5mM glucose within same treatment. Statistical significance was evaluated by unpaired two-tailed Student's t-test. Data are means±SEM.

Figure S3. Related to Figure 3.



<u>Figure S3.</u> The GLP-1R antagonist, Exendin (9-39), has no effect on glucose tolerance and insulin sensitivity in vehicle-treated mice. Related to Figure 3.

(A) 5-hours after food removal, PAHSA-treated mice were i.p. injected with either PBS or 5µg Ex(9-39) 30 minutes prior to the start of an OGTT. Glucose excursion is normalized to baseline glycemia. n=14-16/group. *p<0.05 vs. PAHSA PBS. 5-hours after food removal, vehicle-treated mice were injected with either PBS or 5µg Ex(9-39) intraperitoneally followed by glucose gavage or insulin injection I.P. after 30 minutes for an OGTT (B) or ITT (C). n=14-16/group. *p<0.05 vs. vehicle PBS. (D) Following the same protocol as (B), glycemia, insulin and GLP-1 levels were measured in vehicle-treated mice before and 5 min after glucose gavage. n=14-16/group. *p<0.05 vs. baseline within same treatment, #p<0.05 vs. Vehicle PBS at same time point. Data are means±SEM. (E) GLP-1R reporter assay in HEK293 cells treated with control, 5-PAHSA, 9-PAHSA, hydroxystearic acid, palmitate, or GLP-1. n=3wells/condition. *p<0.05 vs. basal. Statistical significance was evaluated by unpaired two-tailed Student's t-test or two-way ANOVA with Tukey post-hoc tests. Data are means±SEM.

Figure S4. Related to Figure 4.

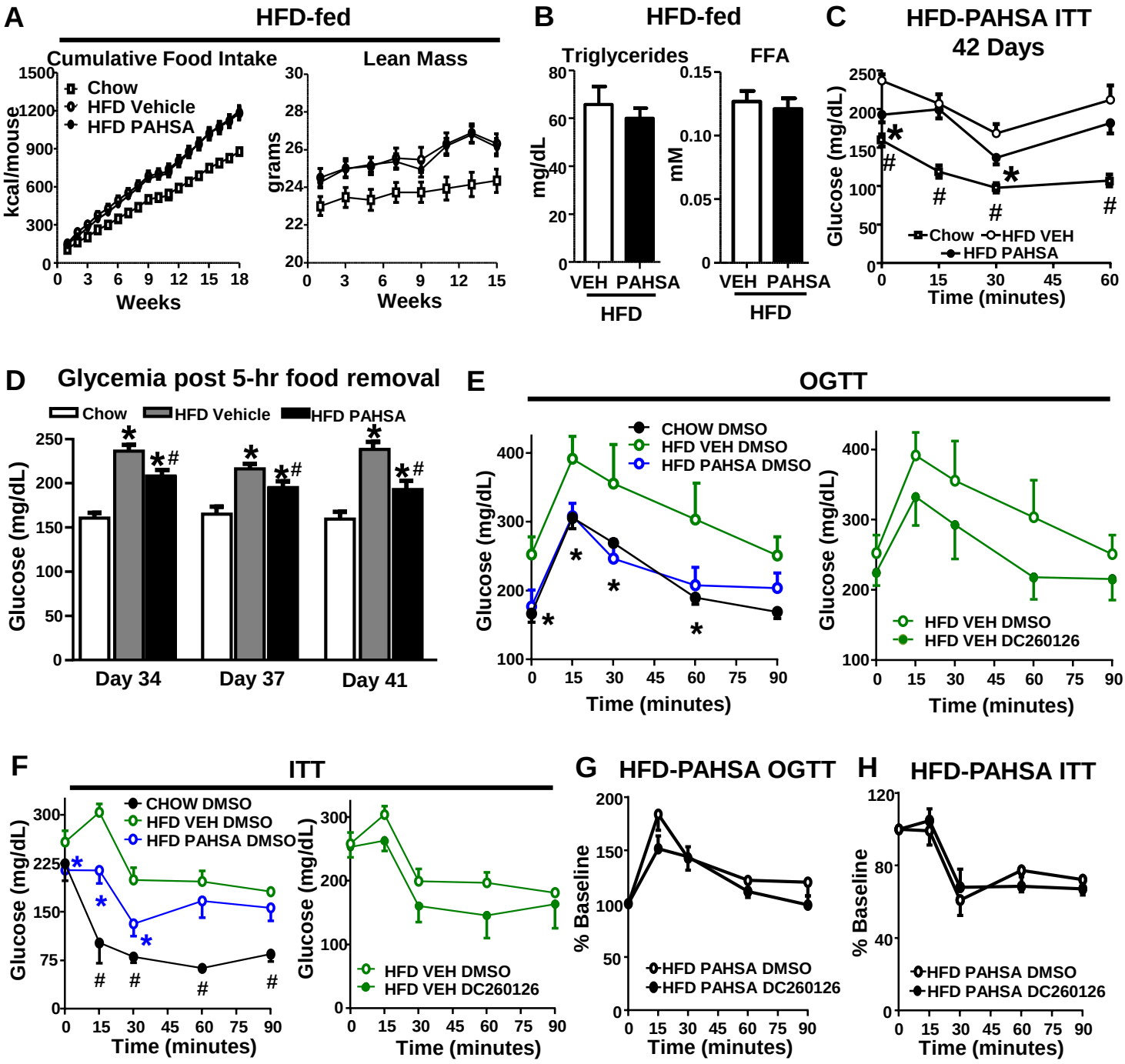


Figure S4. Chronic PAHSA treatment in HFD-fed mice does not affect food intake, lean mass, serum triglycerides or free fatty acids, and improves glycemia and insulin sensitivity. Related to Figure 4.

(A) Cumulative food intake and lean mass in C57bl6 male chow- and HFD-fed mice treated with vehicle or 9-PAHSA via minipumps. n=16/group. (B) Serum triglycerides and free fatty acids (FFA) in vehicle- and PAHSA-treated HFD mice were measured after 4.5 months of treatment. Data are means±SEM. (C) ITT (day 42 of treatment) in vehicle and PAHSA-treated mice. n=8-14/group. *p<0.05 vs. HFD-Vehicle mice; # p<0.05 vs HFD-vehicle and HFD-PAHSA mice. (D) Glycemia 5-hours after food removal in vehicle and PAHSA-treated mice (days 34, 37 and 41 of treatment). n=8-14/group. *p<0.05 vs. Chow-fed mice; # p<0.05 vs HFD vehicle. 5-hours after food removal, mice were injected with either DMSO or DC260126 i.p. and OGTT (E) or ITT (F) was performed 30 minutes later. n=5-6/group. # p<0.05 vs. HFD-vehicle-DMSO and HFD-PAHSA-DMSO; *p<0.05 vs. HFD vehicle DMSO. Data are means±SEM. 5-hours after food removal, PAHSA-treated mice were injected with either DMSO or DC260126 i.p. and OGTT (G) or ITT (H) was performed 30 minutes later. n=5-6/group. Statistical significance was evaluated by unpaired two-tailed Student's t-test or two-way ANOVA with Tukey post-hoc tests. Data are means±SEM.