Supplementary figures

Red Blood Cell β-adrenergic Receptors Contribute to Diet-induced Energy Expenditure by Increasing O_2 Supply

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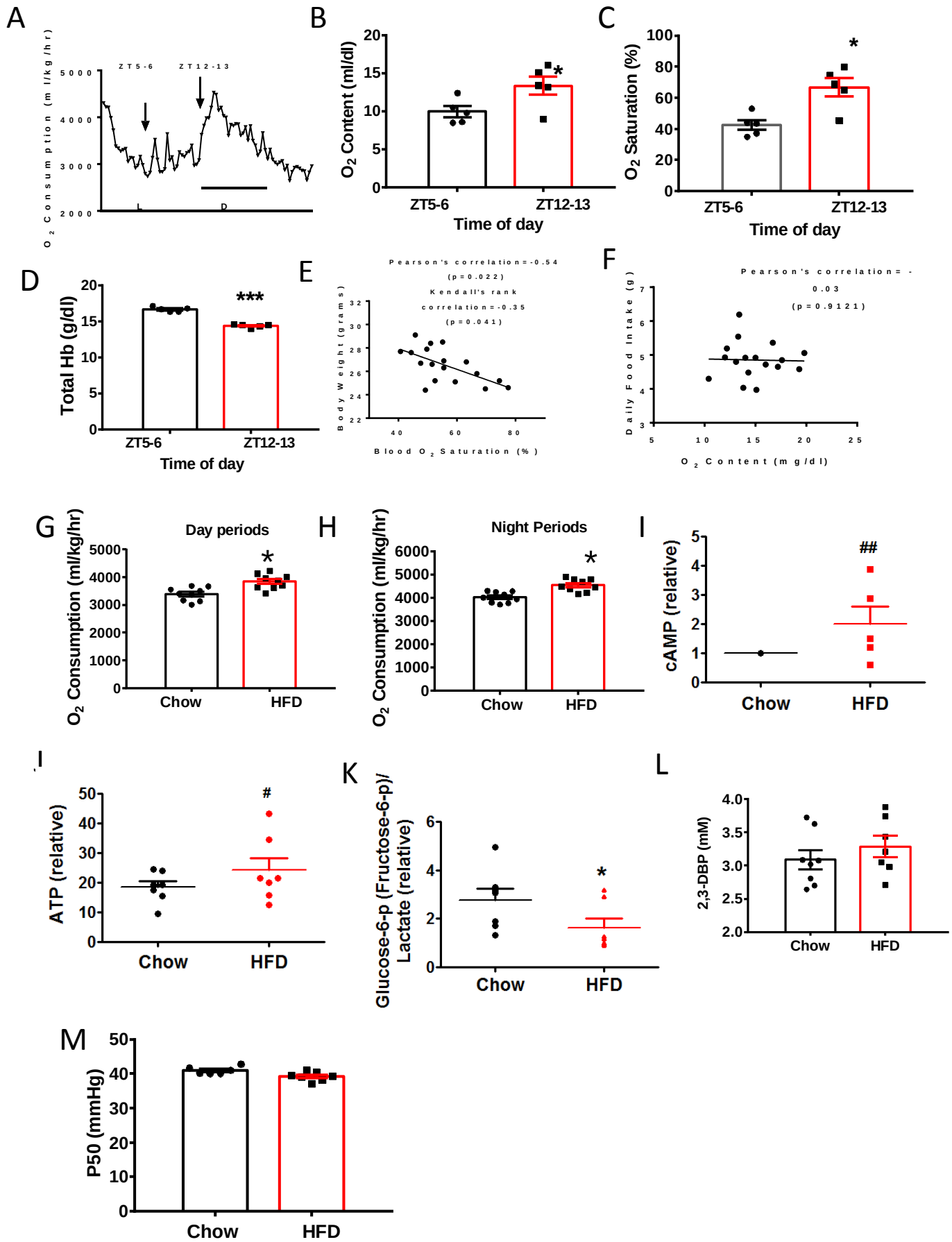

A
B
C
D
E
F
G
H
I
J
K
L
M
ZT5-6
ZT12-13
Time of day
Chow
HFD
Day periods
Night Periods
Pearson's correlation=-0.54
(p=0.022)
Kendall's rank
correlation=-0.35
(p=0.041)
Pearson's correlation=-0.03
(p=0.9121)

Figure S1

Figure S1. Correlation between blood O_2 content and body weight in C57 mice.
(A) O_2 consumption levels of wild type C57 mice during a 24-hour period with time points of ZT5-6 and ZT12-13 indicated (n=5). Data were normalized to body weight per hour. (B-D) blood O_2 content (B), saturation (C), total hemoglobin (D) at day (ZT5-6) and night (ZT12-13) periods (n=5, 8-10wks old males). (E-F) The data was collected from the same cohort of mice described in Figure 1A. Correlation between blood O_2 saturation and body weight (E) and blood O_2 content and daily food intake (F) were conducted by Pearson's correlation tests. P <0.05 was considered significant. (G-H) Energy expenditure changes in response to HFD feeding. Data from the cohort of mice used in Figure 1B showing a significant increase in EE in both day (G) and night periods (H) with HFD feeding. (I-K) cAMP (I) and Glucose-6-phosphate (fructose-6-phosphate)/lactate (K) and ATP levels (J) were measured from isolated RBCs in a cohort of C57 males (8-10 weeks of age, n=7). RBCs were isolated 3 days before and 2 week after HFD switch. The cAMP readings from C57 mice had a large baseline variation, and for a better comparison, all readings at the Chow diet condition was normalized to 1. In addition, 2 samples were removed from analysis in the cAMP measurement because these samples showed a more than 20 folds increase in HFD conditions compared to Chow, which is out of range of normal distribution. (L-M) RBCs were collected from the same cohort of mice described in Figures 1B-1F and used for the measurements of 2,3-DPG (n=7-8/group) (L) and of P50 (n=6-7) (M). All data are presented as mean ± SEM; *p<0.05; ***p<0.001; $^{\#}$p=0.07; and $^{\#\#}$P=0.06, paired Student's t tests.

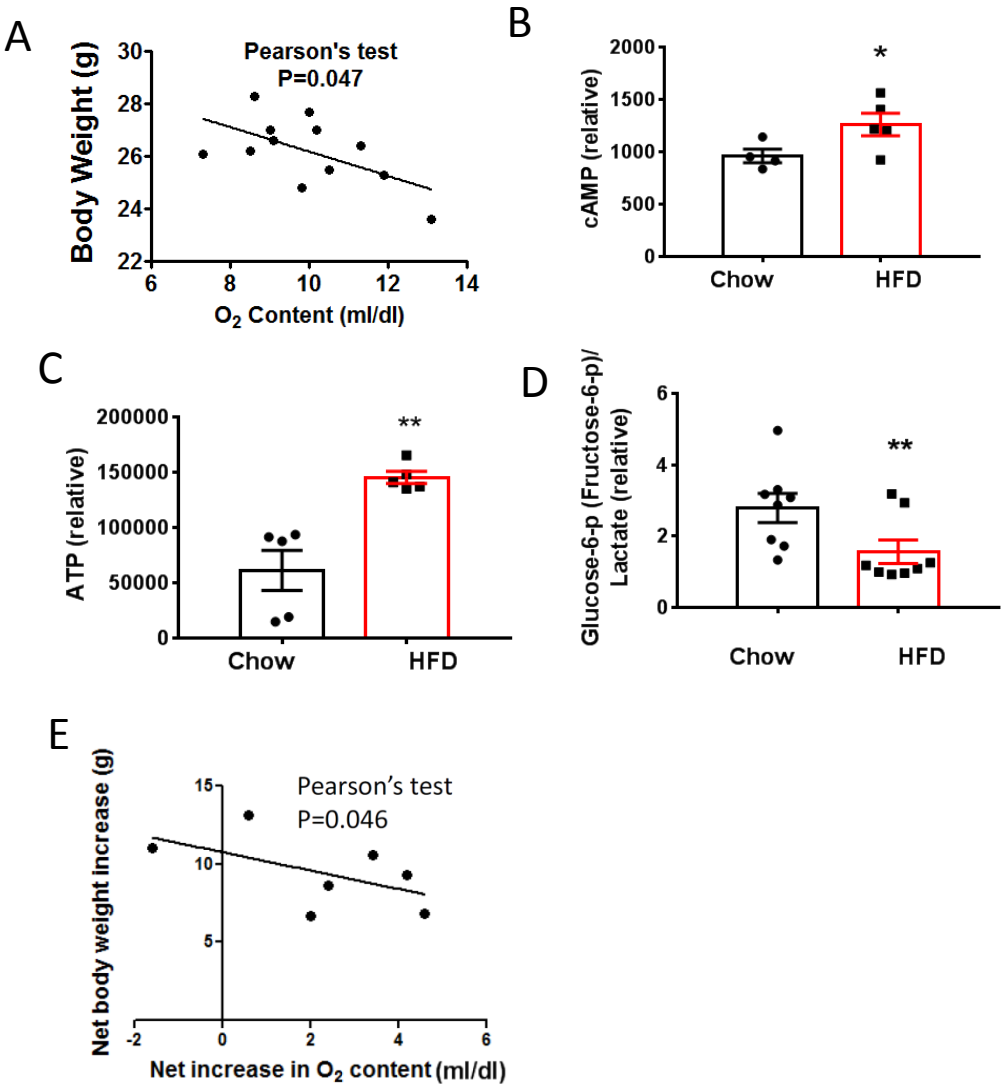


Figure S2. Correlation between blood O_2 content and body weight in FVB mice.
(A) Correlation of body weight and O_2 content measured in a cohort of male FVB mice at the age of 8 weeks of age. (B-C) cAMP (B) and ATP (C) were measured from a cohort of adult male FVB mice (7- weeks of age) in RBCs obtained from tail blood 3 days before and 4 weeks after switch to HFD (n=5). (D) Glucose-6-p (Fructose-6-p)/lactate was measured in another cohort of adult male FVB mice (n=8) with the same HFD treatment. (E) Using the same experimental design as in Fig. 1H, regression analysis was tested between net body weight changes and net increases in O2 content in a cohort of FVB mice (n=7 males). These mice were reared chow and switched to HFD at 7-8 weeks of age. Changes in body weight and O2 contents were collected 3 days before diet switch and 4 weeks after diet switch. All data are presented as mean ± SEM; *p<0.05, **p<0.01, paired Student's t tests.

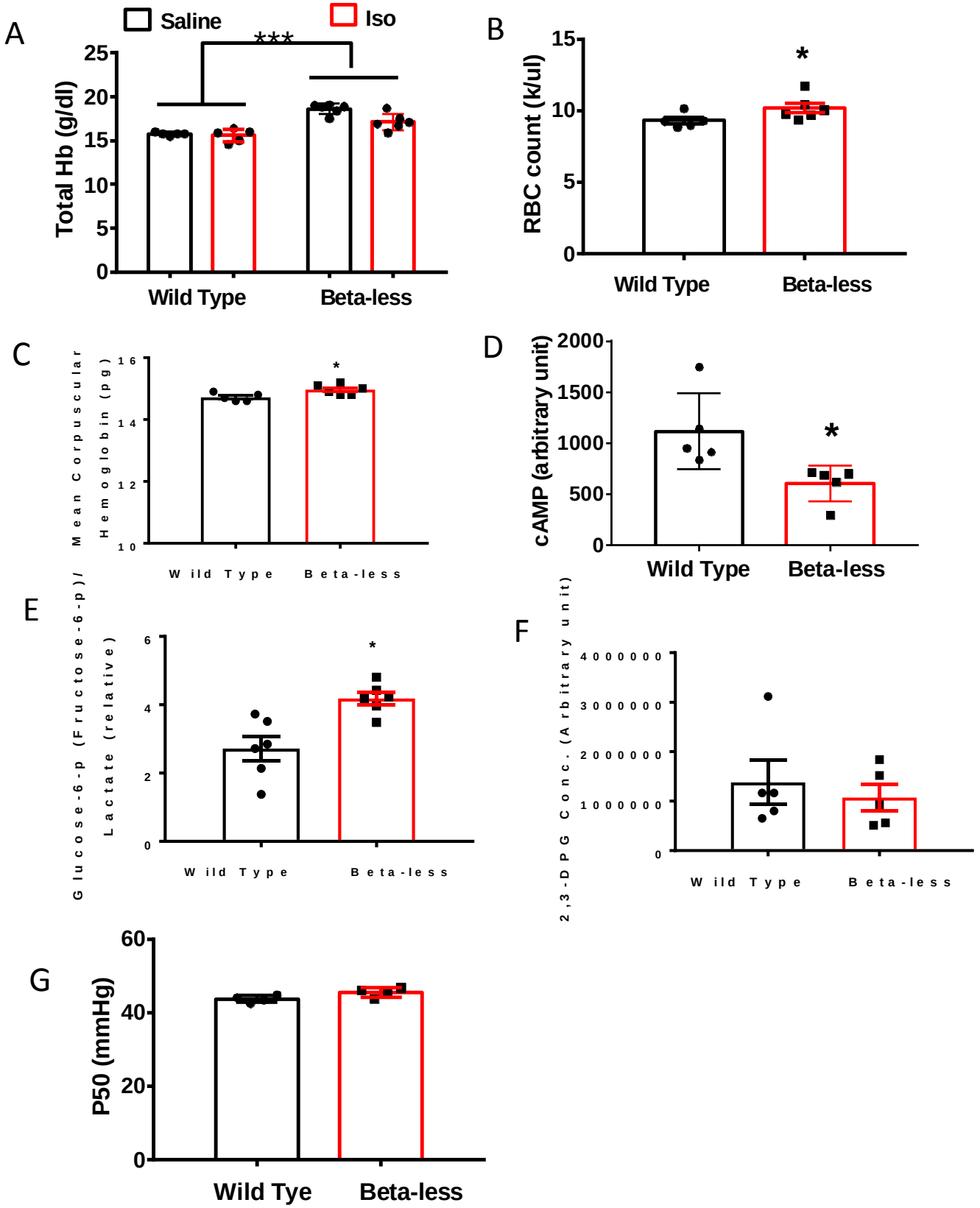
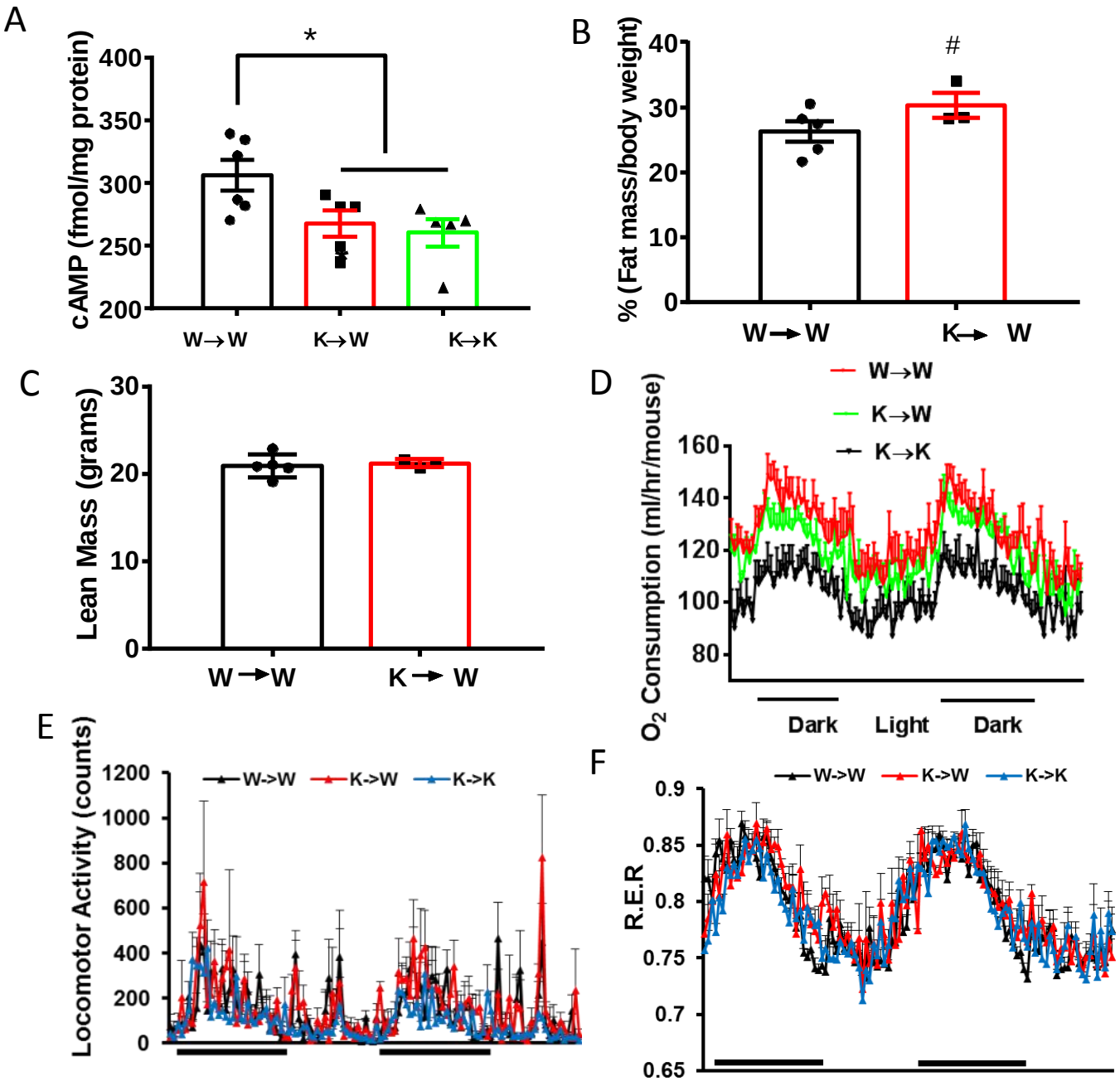

A
Saline
Iso
***
Total Hb (g/dl)
25
20
15
10
5
0
Wild Type
Beta-less
B
RBC count (k/ul)
15
10
5
0
*
Wild Type
Beta-less
C
Mean Corpuscular Hemoglobin (pg)
16
14
12
10
*
Wild Type
Beta-less
D
cAMP (arbitrary unit)
2000
1500
1000
500
0
*
Wild Type
Beta-less
E
Glucose-6-p (Fructose-6-p)/ Lactate (relative)
6
4
2
0
*
Wild Type
Beta-less
F
2,3-DPG Conc. (Arbitrary unit)
40000000
30000000
20000000
10000000
0
Wild Type
Beta-less
G
P50 (mmHg)
60
40
20
0
Wild Tye
Beta-less

Figure S3

Figure S3. Effects of β-AR deficiency in BM cells on RBCs .
RBCs were obtained from Beta-less mice and controls for the measurements (n=5-8, males, 8-10 weeks of age). (A) Total blood hemoglobin levels measured before and after Iso administration in WT FVB mice and Beta-less mice. RBC number (B), mean corpuscular hemoglobin (C), cAMP (D), glucose-6-p (fructose-6-p)/lactate (E), 2,3-DPG (F) and P50 (G). Total Hb was measured by Avoximeter, RBC number and mean corpuscular hemoglobin was measured by a whole blood count, cAMP, glucose-6-p(fructose-6-p), lactate and 2,3-DPG were measured by the metabolomics core at Dr. Feng Li at Baylor College of Medicine. P50 was measured by a hemox analyzer by Dr. Yang Xia. All data are presented as mean ± SEM; *$p<0.05$; ***$p<0.005$, one way ANOVA (A) or unpaired student's t tests (B-E).



Figures S4. Effects of β-AR deficiency in BM cells on metabolism.
Data was obtained from W→W, K→W and K→K mice (12-16 weeks of age, n=5-7, males) described in Fig. 2. From these 3 groups of mice, we measured cAMP levels from isolated RBCs (A), fat mass (B), lean mass (C), O_2 consumption analyzed per animal (D), locomotion activity (E) and RER (F). Data presented in D-F were measured by CLAMS. All data are presented as mean ± SEM; *p<0.05, #p=0.07. One way ANOVA (A) or unpaired Student's t tests (B).

Figure S4

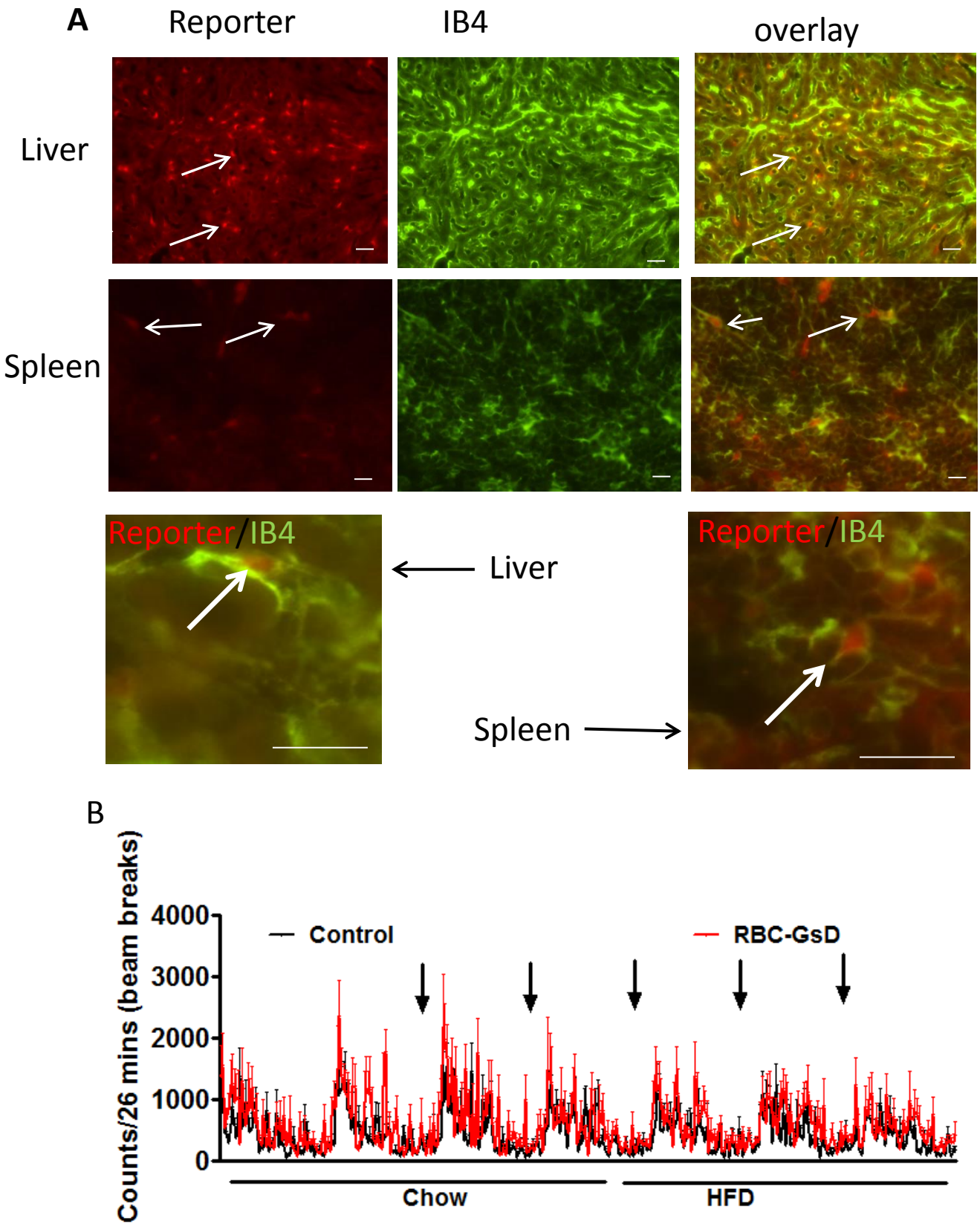

A
Reporter
IB4
overlay
Liver
Spleen
Reporter/IB4
Liver
Spleen
Reporter/IB4
B
Counts/26 mins (beam breaks)
4000
3000
2000
1000
0
Control
RBC-GsD
Chow
HFD

Figure S5

Figure S5. EpoR-Cre expression pattern and the effect of RBC Gs on locomotion.
(A) EpoR-Cre::Ai9 mice were used and main metabolic tissues were screened by potential Cre expression. Immunostaining for IB4 (green), an endothelial marker, was performed on tissues that showed Cre-mediated expression of tdTomato (RFP). Distinct RFP positive structures were found in both liver and spleen, in addition to the brain shown in Figure 3. These structures were found to be confined within IB4-expressing vessels (white arrows), suggesting that they were RBCs in nature. The confinement was clearly shown in the high-magnification pictures of liver and spleen (arrow in bottom panels). (B) Locomotion was monitored during the transition from Chow (first 3 days) to HFD (last 3 days) with CNO administration showing that there was no change in locomotion between genotypes or drugs. Scale bar: 50μM. Arrows in B indicate CNO administration.

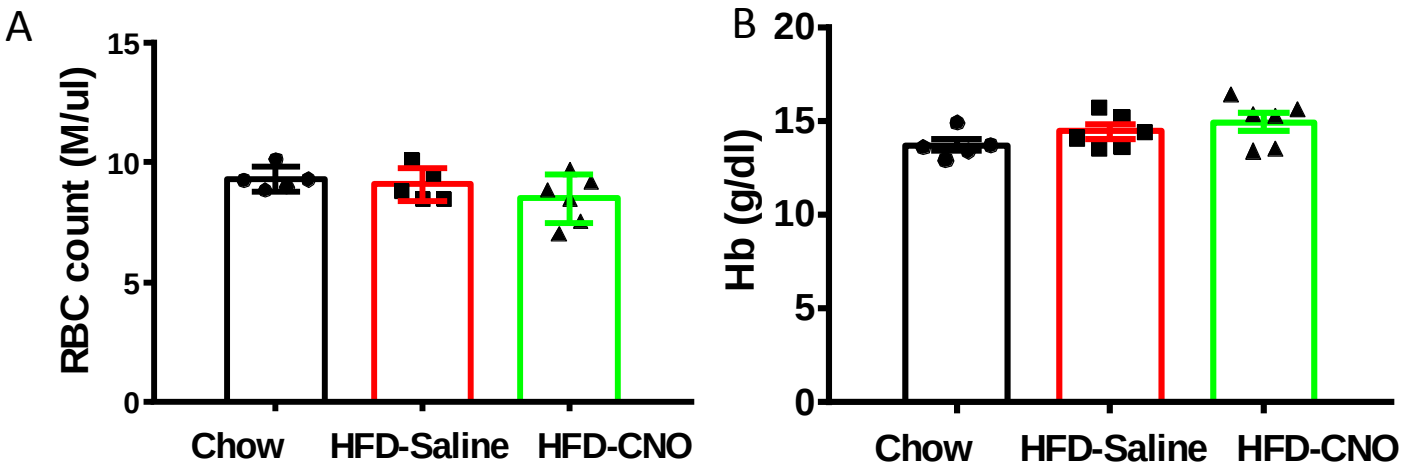


Figure S6. Effects of RBC Gs-cAMP signaling activation on RBCs and Hb levels.
Effect of daily i.p. CNO (1mg/kg b.w., 10:00 and 17:00) for 2 weeks on the mice (control and RBC-Gs mice) presented in Figure 5. A whole blood count was performed from these mice (chow fed controls, RBC-GsD mice fed HFD treated with saline and RBC-GsD mice fed HFD treated with CNO, n=5-6 males). No changes were noted in RBC count (A) or Hb levels (B). All data are presented as mean ± SEM. One way ANOVA tests.

Figure S6