Supplementary Information

Supplemental Figure Legend

Figure S1 is related to Figure 1

Supplementary Figure 1- Male C57BL/6J mice are sensitizing to diet-induced obesity and insulin resistance and increasing S6 activities in HFD-fed C57BL/6J mice.

A. Fat mass measurement in *wild-type* C57BL/6J male and female mice on a normal chow and HFD at 6 months of age.

B. Plasma glucose measurement in 6-hr-fasting *wild-type* C57BL/6J male and female mice on a normal chow and HFD at 6 months of age.

C. Insulin challenge assay in 6-hr-fasting *wild-type* C57BL/6J male and female mice on a HFD at 6 months of age (n=44).

D. Plasma triglyceride measurement in 6-hr-fasting *wild-type* C57BL/6J male and female mice on a normal chow and HFD at 6 months of age.

E. Western blot of phosphorylated S6 at Ser 235/236 and total S6 protein expression in quadriceps muscle, liver and visceral fat from normal-diet-fed and HFD-fed *wild-type* mice at 6 months of age.

F. Quantification of western blot of phosphorylated S6 at Ser 235/236 normalized with total S6 expression, relative to normal-diet-fed samples.

All graphs are plotted as means ± SEM of n, number of mice used in each analysis. Number of samples analyzed indicated in figure. P values were calculated by a two-way ANOVA [(A), (B), (D) and (F)], and two-way ANOVA for repeated measures [(C)] with Bonferroni post-tests to compare replicate means by row. P value of the cross comparison from the other groups was labeled *P<0.05;**P<0.01; ***P<0.001.

Figure S2 is related to Figure 2

Supplementary Figure 2- Decreasing 4E-BP1 expression in HFD-fed male C57BL/6J mouse tissues and acute inflammation treatment decreases 4E-BP1 expression in wild-type MEFs.

A. Western blot of 4E-BP1 and housekeeping genes (βactin and αtubulin) protein expression in quadriceps muscle, and visceral fat from normal-diet-fed and HFD-fed *wild-type* male mice at 6 months of age. Each lane represents individual mice fed with indicated diet. 4EBP1* indicates longer exposure.

B. Quantification of western blot of 4E-BP1expression normalized with housekeeping genes relative to normal diet fed samples.

C. Quantification of western blot of 4E-BP1expression normalized with housekeeping gene, relative to male from normal diet fed samples in quadriceps muscle. P values were calculated by one-tailed unpaired student's t-test.

D. Quantification of western blot of phosphorylated 4EBP1 at Thr 37/46 normalized with total 4EBP1 expression, relative to normal-diet-fed samples.

E. Western blot of 4E-BP1 and housekeeping genes (αtubulin) protein expression in visceral fat from normal-diet-fed and HFD-fed *wild-type* male mice at 6 months of age. Each lane represents individual mice fed with indicated diet.

F. Quantification of western blot of 4E-BP1expression normalized with housekeeping gene, HSP90, or phosphorylated JNK at Thr183/Tyr185 normalized with total JNK expression relative to LPS non-treated samples. (n=4)

G. *Wild-type* MEFs were treated with LPS at indicated time intervals. Samples of cell lysates were analyzed by Western blot of 4E-BP1, pJNK (indicated inflammation), JNK and HSP90 (loading control).

All graphs are plotted as means ± SEM of n, number of mice used in each analysis. Number of samples analyzed indicated in figure. P values were calculated by a two-way ANOVA with Bonferroni post-tests to compare replicate means by row.

Figure S3 is related to Figure 3

Supplementary Figure 3- Generation of *4EBP1-OE* transgenic mice.

A. Construct of *4E-BP1* transgenic allele. The expression of transgenic *4E-BP1* is under the control of the chicken *β –actin* promoter.

B. Western blot of 4E-BP1 protein expression in quadriceps muscle, liver, visceral fat and brown adipose tissues from normal-diet-fed male mice at 2 months of age.

C. Western blot of phosphorylation of 4E-BP1 at Ser 65 in visceral fat of *4EBP1-OE* transgenic and *wild-type* male mice before (-) or after (+) insulin stimulation on a normal chow at 2-months of age.

D. Western blot of cap binding assay to analyze eIF4E-eIF4G complex formation in skeletal muscle. The translation initiation complex was pull down by the cap analog m7GTP-sepharose and western blotted with antibodies against eIF4G, eIF4E, or 4E-BP1.

E. Quantification of cap binding assays on eIF4G level, which associated with translation initiation complex normalized to control. P values were calculated by one-tailed unpaired student's t-test.

F. Representative pictures of Oil-red stained liver section and Hematoxylin & Eosin stained sections on skin, visceral fat, brown adipose and quadriceps muscle from 6-month-old mice fed a normal chow, scale bar = 100μm. (n=8 per group)

G. Body weight measurement in *4EBP1-OE* transgenic and *wild-type* mice on a normal chow (n=7~11).

H. Plasma glucose measurement in 6-hr-fasting *4EBP1-OE* transgenic and *wild-type* mice on a normal chow. P values were calculated by a two-way ANOVA with Bonferroni post-tests to compare replicate means by row.

All graphs are plotted as means ± SEM of n, number of mice used in each analysis.

Figure S4 is related to Figure 3

Supplementary Figure 4- Measurement of metabolic homeostasis of *4EBP1-OE* transgenic and *wild-type* mice at 6 month-old of age.

A. Glucose tolerance assay in 6-hr-fasting *4EBP1-OE* transgenic and *wild-type* mice (n=7-11).

B. Insulin challenge assay in 6-hr-fasting *4EBP1-OE* transgenic and *wild-type* mice (n=4).

C. Fat mass measurement in fasting *4EBP1-OE* transgenic and *wild-type* mice.

D. Plasma triglyceride measurement in 6-hr-fasting *4EBP1-OE* transgenic and *wild-type* mice.

E. Quantification of adipose cell size in visceral fat of *4EBP1-OE* transgenic and *wild-type* mice (n=2-5).

F. Analysis of the mean adipose cell size in visceral fat of *4EBP1-OE* transgenic and *wild-type* mice from **C.** (n=2-5).

G. Quantification of western blot of UCP1expression normalized with housekeeping gene, HSP90, relative to male samples in brown adipose tissues.

H. Food intake was measured in 3-night period of time in *4EBP1-OE* transgenic and *wild-type* mice on a HFD at 6 month-old of age.

I. Serum IL-6 was measured in *4EBP1-OE* transgenic and *wild-type* male mice on HFD.

J. Serum Insulin measurement in 6-hr-fasting *4EBP1-OE* transgenic and *wild-type* mice on HFD.

K. Insulin challenge assay in 6-hr-fasting *4EBP1-OE* transgenic and *wild-type* mice on a HFD at 6 month-old of age (n=9).

L. The measurement of area under curve from (K).

All graphs are plotted as means ± SEM of n, number of mice used in each analysis. Number of samples analyzed indicated in figure. P values were calculated by a two-way ANOVA for repeated measures [(A) (B), and (K)] and two-way ANOVA [(C), (D), (E), (F), (H), (J), and (L)] with Bonferroni post-tests to compare replicate means by row, and P values were calculated by one-tailed unpaired student's t-test [(G) and (I)]. P value of the cross comparison from the other groups was labeled *P<0.05;**P<0.01; ***P<0.001.

Figure S5 is related to Figure 3

Supplementary Figure 5- Male *4EBP1-OE* transgenic mice protect from diet induced metabolic dysfunction.

A. Representative pictures of Oil-red stained liver section characterizing lipid accumulation, Hematoxylin & Eosin stained adipose sections on skin, inguinal fat, brown adipose, and visceral fat, and F4/80 IHC stained visceral fat detecting macrophage infiltration from 6-month-old mice fed a HFD, scale bar = 100 μm (n=8 per group).

B. Quantification of western blot of FGF21 expression normalized with housekeeping gene, HSP90, relative to *wild-type* samples in livers from HFD-fed male mice at 6-months of age. Graph is plotted as means ± SEM of n, number of mice used in each analysis. Number of samples analyzed indicated in figure. P values were calculated by one-tailed unpaired student's t-test.

C. Western blot of phosphorylation of AKT1 at Ser 473 and total AKT1 protein expression in liver of *4EBP1-OE* transgenic and *wild-type* mice before (-) or after (+) insulin stimulation on a normal chow or HFD at 6 month-old of age.

D. Western blot of phosphorylation of AKT1 at Ser 473 and total AKT1 protein expression in skeletal muscle of *4EBP1-OE* transgenic and *wild-type* mice before (-) or after (+) insulin stimulation on a normal chow or HFD at 6 month-old of age.

E. Western blot of phosphorylation of AKT1 at Ser 473 and total AKT1 protein expression in visceral fat of *4EBP1-OE* transgenic and *wild-type* female mice before (-) or after (+) insulin stimulation on a normal chow or HFD at 6 month-old of age.

F. Quantification of western blot of phosphorylation AKT1 at Ser 473 normalized with total AKT1 expression, relative to paired fasting sample. n=3~6.

Figure S6 is related to Figure 3

Supplementary Figure 6- The assessment of other mTORC1 target signaling in HFD-fed *wild-type* and *4EBP1-OE* transgenic mice

A. Quantification of western blot of phosphorylated S6K1 at Thr 389 normalized with total S6K1 expression, relative to *wild-type* samples in quadriceps muscle and visceral fat from HFD-fed mice at 6-months of age.

B. Quantification of western blot of phosphorylated S6 at Ser 235/236 normalized with total S6 expression, relative to *wild-type* samples in liver and quadriceps from HFD-fed mice at 6-months of age.

C. Quantification of western blot of phosphorylated PRAS40 at Ser 183 normalized with total PRAS40 expression, relative to relative to normal diet fed samples in *wild-type* mouse visceral fat.

D. Quantification of western blot of phosphorylated PRAS40 at Ser 183 normalized with total PRAS40 expression, relative to *wild-type* samples from HFD-fed male mice at 6-months of age.

E. Quantification of western blot of DEPTOR expression normalized with housekeeping gene, HSP90, relative to normal diet fed samples in male *wild-type* mouse tissues.

F. Quantification of western blot of DEPTOR expression normalized with housekeeping gene, HSP90, relative to *wild-type* samples in visceral fat from HFD-fed male mice at 6-months of age. P values were calculated by one-tailed unpaired student's t-test.

All graphs are plotted as means ± SEM of n, number of mice used in each analysis. Number of samples analyzed indicated in figure. P values were calculated by a two-way ANOVA with Bonferroni post-tests to compare replicate means by row.

Figure S7 is related to Figure 4

Supplementary Figure 7- Generation of *4E-BP1* double transgenic mice.

A. Western blot of 4E-BP1 protein expression in quadriceps muscle from normal-diet-fed male *Tg-4EBP1wt-muscle* mice at 2-months of age.

B. Western blot of 4E-BP1 protein expression in visceral fat muscle from normal-diet-fed male *Tg-4EBP1wt-fat* mice at 2-months of age.

C. Body weight measurement in *Tg-4EBP1wt-fat* and *Tg-4EBp1wt-muscle* female mice on a normal chow and HFD (n=8-16).

D. Fasting glucose measurement in *Tg-4EBP1wt-fat* and *Tg-4EBp1wt-muscle* mice on a normal chow and HFD at 6-months of age (n=8-16).

E. Plasma glucose measurement in 6-hr-fasting *Tg-4EBP1wt-fat* and *Tg-4EBp1wt-muscle* female mice on a normal chow and HFD 6-months of age (n=8-16).

F. Insulin challenge assay in 6-hr-fasting *Tg-4EBP1wt-fat* and *Tg-4EBp1wt-muscle* female mice on a HFD 6-months of age (n=8-16).

G. Western blot of 4E-BP1-Serine 65 phosphorylation in visceral fat muscle from normal-diet-fed male *Tg-4EBP1wt-fat* mice 2-months of age.

H. Western blot of 4E-BP1 Serine 65 phosphorylation in quadriceps muscle from normal-diet-fed male *Tg-4EBP1wt-muscle* mice 2-months of age.

All graphs are plotted as means ± SEM of n, number of mice used in each analysis. Number of samples analyzed indicated in figure. P values were calculated by a two-way ANOVA [(D)] and a two-way ANOVA for repeated measures [(E), and (F)] with Bonferroni post-tests to compare replicate means by row. [$P<0.001$, NCD vs HFD cross genotype]

Figure S8 is related to Figure 5

Supplementary Figure 8- Metabolic parameters of *4EBP1-OE* transgenic and *wild-type* mice under a normal chow during aging.

A. Lean mass measurement in *4EBP1-OE* transgenic and *wild-type* mice on a normal chow during aging.

B. Plasma leptin measurement in *4EBP1-OE* transgenic and *wild-type* mice on a normal chow at 6 month of age.

C. Plasma triglyceride measurement in 6-hr-fasting *4EBP1-OE* transgenic and *wild-type* mice on a normal chow at 6 months of age.

D. Food consumption measured in 3 day-night period of time in 6 month-old and 20 month-old *4EBP1-OE* transgenic and *wild-type* mice fed a normal chow.

E. Home cage activity in 3 day-night period of time in 6 month-old and 20 month-old *4EBP1-OE* transgenic and *wild-type* mice fed a normal chow.

F. Respiratory exchange ratio in 3 day-night period of time in 6 month-old and 20 month-old *4EBP1-OE* transgenic and *wild-type* mice fed a normal chow.

G. Quantification of western blot of phosphorylated S6K1 at Thr 389 normalized with total S6K1 expression, relative to *wild-type* samples in quadriceps muscle and visceral fat from 24-months of age mice.

H. Quantification of western blot of phosphorylated S6 at Ser 235/236 normalized with total S6 expression, relative to *wild-type* samples in liver from 24-months of age mice.

I. Quantification of western blot of phosphorylated IRS1 at Ser 636/639 normalized with total IRS1 expression or IRS1 expression normalized with housekeeping gene, HSP90, relative to *wild-type* samples in visceral fat from 24-months of age mice.

J. Quantification of western blot of puromycin incorporation in total protein normalized with housekeeping gene, HSP90, relative to *wild-type* samples from 24-months of age mice.

K. Fat mass measurement normalized with body weight in *wild-type* C57BL/6J male and female mice.

All graphs are plotted as means ± SEM of n, number of mice used in each analysis. P values were calculated by a two-way ANOVA with Bonferroni post-tests to compare replicate means by row. P value of the cross comparison from the other groups was labeled *P<0.05;**P<0.01; ***P<0.001.

Figure S9 is related to Figure 5

Supplementary Figure 9- The assessment of aging *4EBP1-OE* transgenic mouse fat and skeletal muscle tissues.

A. Quantification of western blot of phosphorylated PRAS40 at Ser 183 normalized with total PRAS40 expression, relative to relative to *wild-type* samples in 24-months of age mouse visceral fat.

B. Plasma FGF21 measurement in 6-hr-fasting *4EBP1-OE* transgenic and *wild-type* mice on a normal chow at 18 months of age.

C. Representative pictures of Hematoxylin & Eosin stained sections on brown adipose, subcutaneous white adipose, visceral fat, inguinal fat, and retroperitoneal white adipose from 24-month-old mice on a normal chow, scale bar = 100μm. (n=8-10 per group)

D. Real-time PCR analysis of thermogenesis gene expression in subcutaneous white adipose tissues from 24-month-old mice. Fold induction was normalized to *wild-type* mouse samples.

E. Real-time PCR analysis of *Ucp-1* and *Pgc-1α* mRNA expression in brown adipose tissues from 24-month-old mice. Fold induction was normalized to *wild-type* male mouse samples.

F. Quantification of percentage of central nuclei in 24 month-old mouse soleus muscles.

G. Representative pictures of Hematoxylin & Eosin stained sections on soleus muscle from 24-month-old mice fed a normal chow, scale bar = 100μm. (n=4-5 per group)

H. Representative pictures of Hematoxylin & Eosin and Oil-Red stained sections to detect lipid accumulation on quadriceps muscle from 24-month-old mice fed a normal chow, scale bar = 100μm (n=8-10 per group). Blue arrows indicated vacuolated fibers.

All graphs are plotted as means ± SEM of n, number of mice used in each analysis. Number of samples analyzed indicated in figure. P values were calculated by a two-way ANOVA with Bonferroni post-tests to compare replicate means by row. P value of the cross comparison from the other groups was labeled *P<0.05;**P<0.01; ***P<0.001.

Figure S10 is related to Figure 6

Supplementary Figure 10- Increasing S6K1 activity in aging female C57BL/6J mouse liver.

A. Western blot of phosphorylated S6 at Ser 235/236 and total S6 protein expression in liver.

B. Quantification of western blot of phosphorylated S6 at Ser 235/236 normalized with total S6 expression, relative to 6-months old mouse samples. Number of samples analyzed indicated in figure and results are presented as means ± SEM. P values were calculated using two-way ANOVA with Bonferroni post-tests.

Supplemental Experimental Procedures

Cell Culture

For the lipopolysaccharide (LPS) treatment, passage 5 *wildtype* mouse embryonic fibroblasts (MEFs) were plated and serum deprived overnight in Dulbecco's modified Eagle's medium (DMEM). The next day, they were treated with 0.5 μg/ml LPS (Sigma) and harvested at the indicated time point.

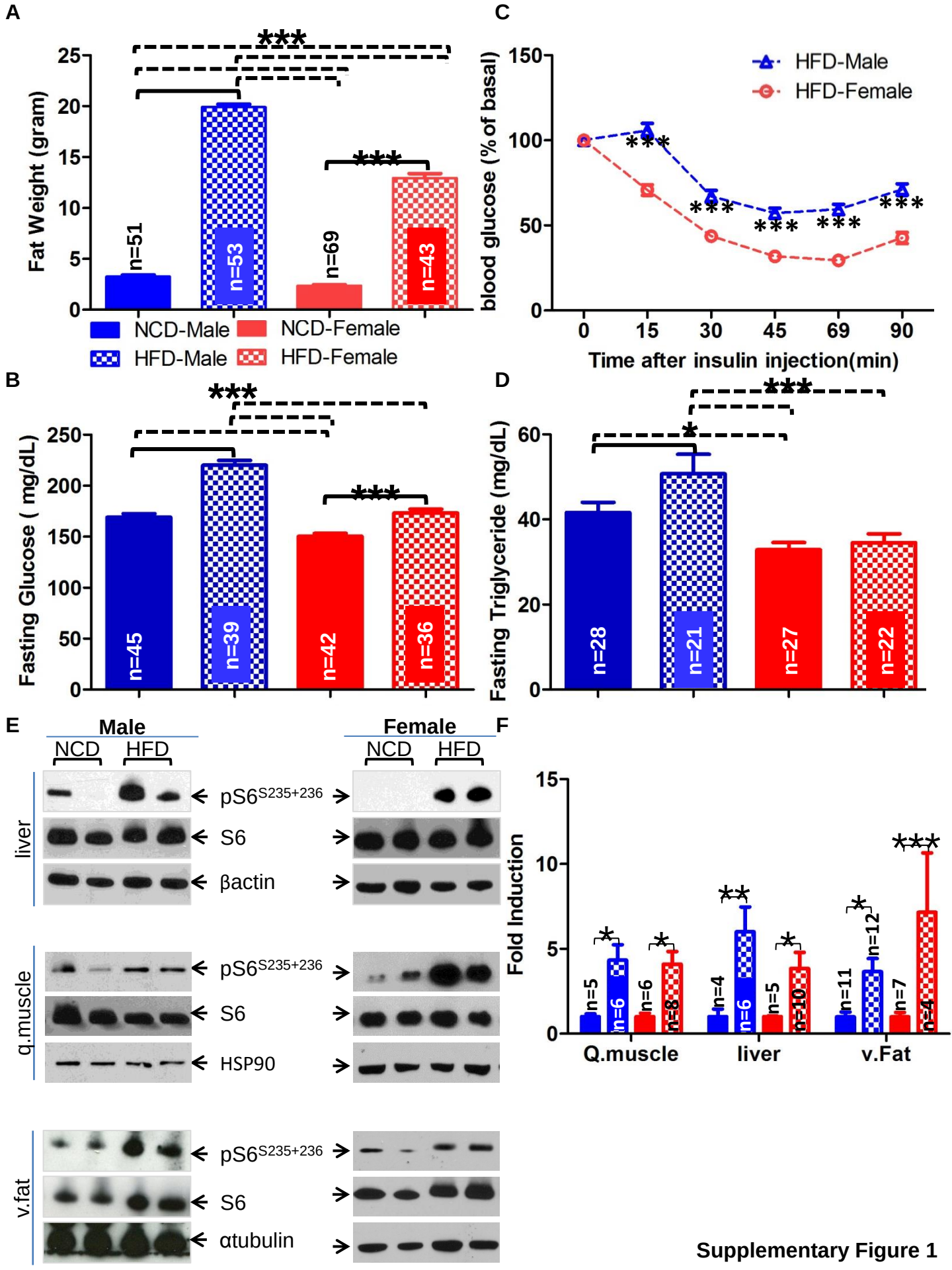
Cap-binding assay and immunoprecipitation

Tissues were homogenized in cold buffer A (10mM Tris pH7.5, 150mM KCl, 4mM MgCl2 and 1mM EDTA and 1% NP-40) with protease inhibitor cocktail (Roche 04693124001) and phosphatase inhibitor cocktail II and III (Sigma P5726 and P0044). For Cap-binding assay, 1.2mg of tissue lysates were incubated overnight at 4^{o}C with 50μl of the mRNA cap analogue. m7GTP –agarose (Jena Bioscience) in buffer A. The complex was washed once with buffer A. Protein complexes were eluted using 2x sample buffer, and evaluated in western blot.

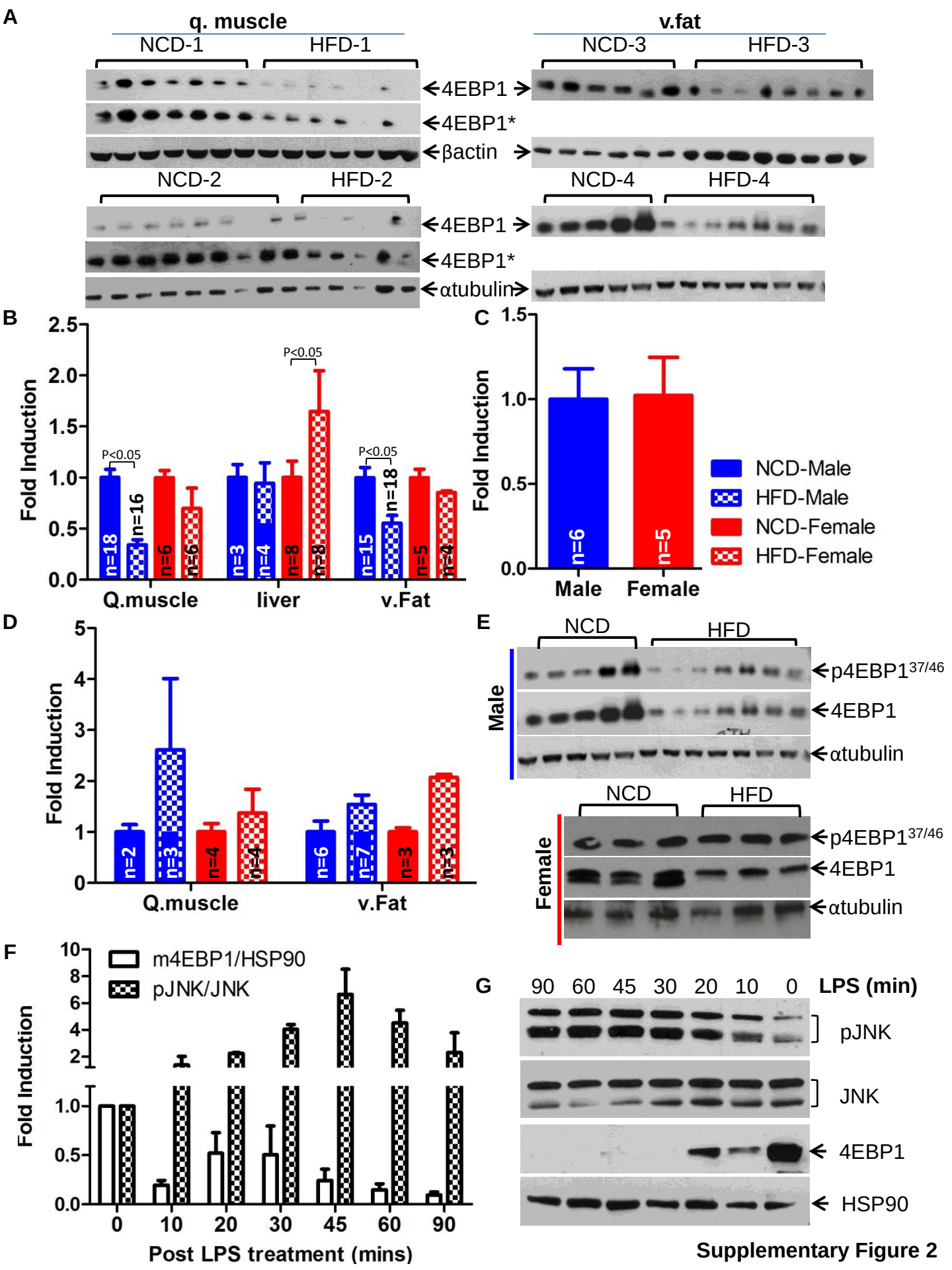
Protein synthesis assay

A non-isotope labeled protein synthesis assay was performed as previously described using theSUnSET method (84, 85). Puromycin (0.04 uM/g puromycin) was injected 30 minutes prior to harvesting. Protein was extracted and analyzed as stated above. Total protein was assessed

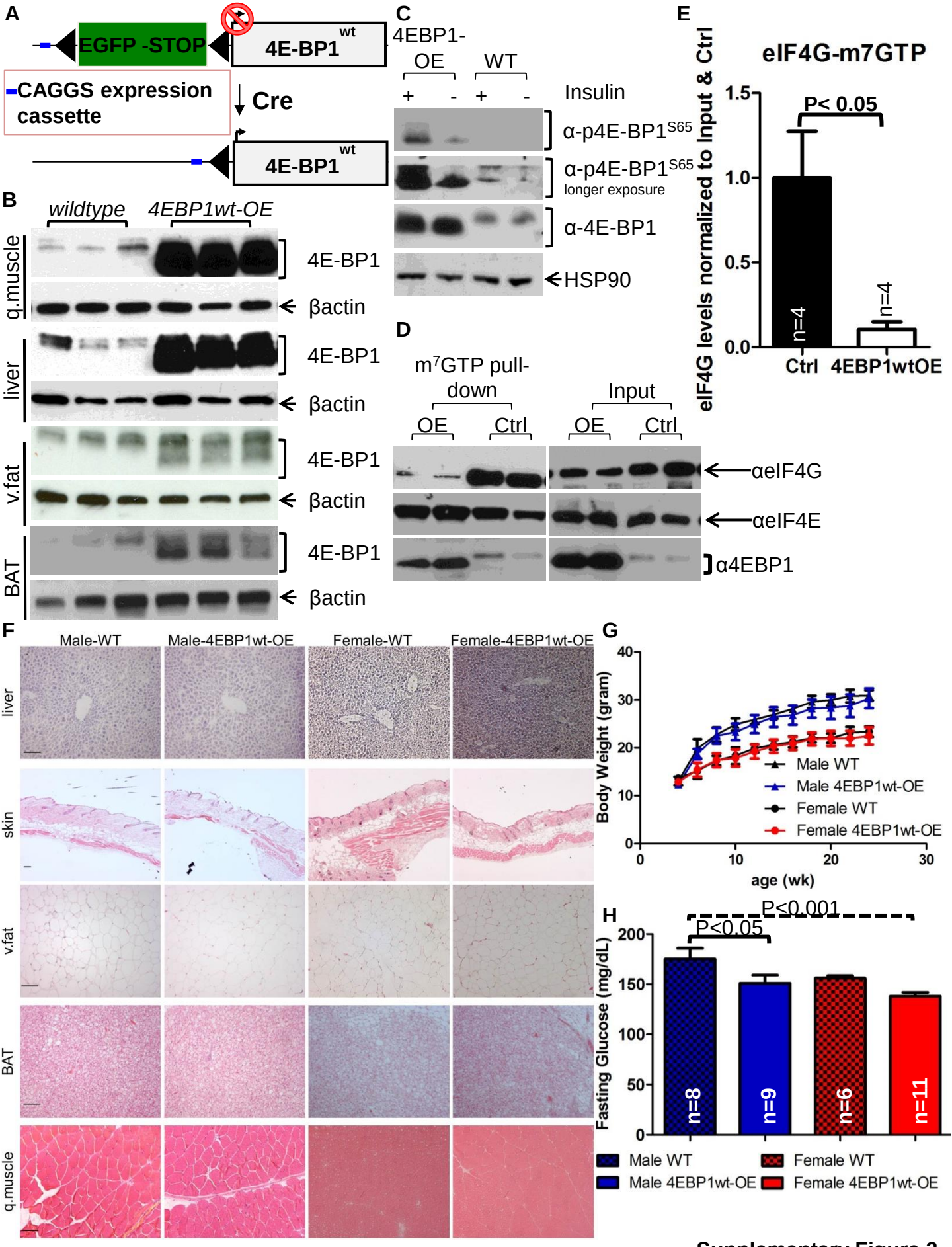
using the loading control, HSP90 and newly synthesized puromycin incorporated protein was detected by puromycin antibody (Gift from Dr. Philippe Pierre in INSERM).



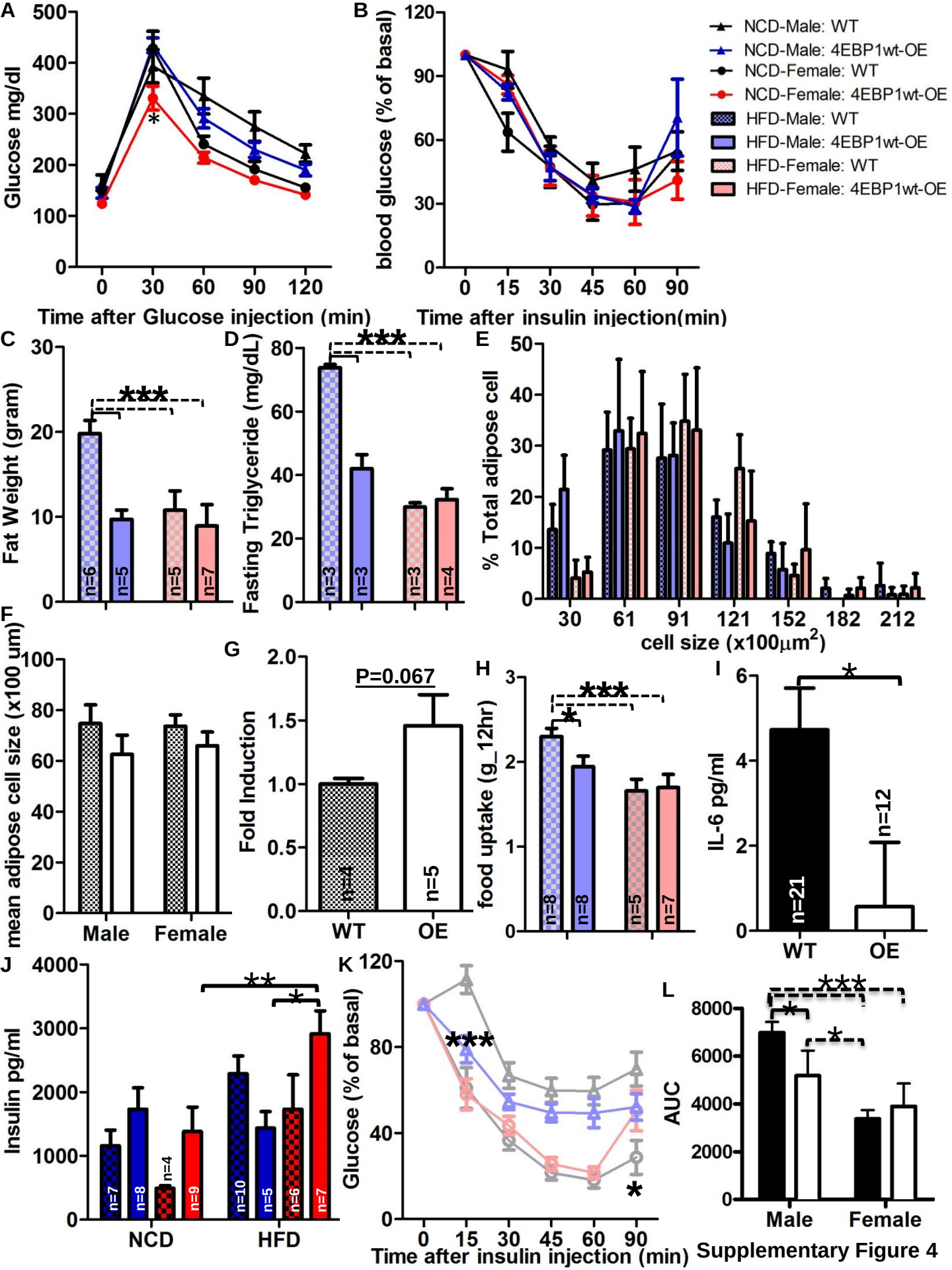
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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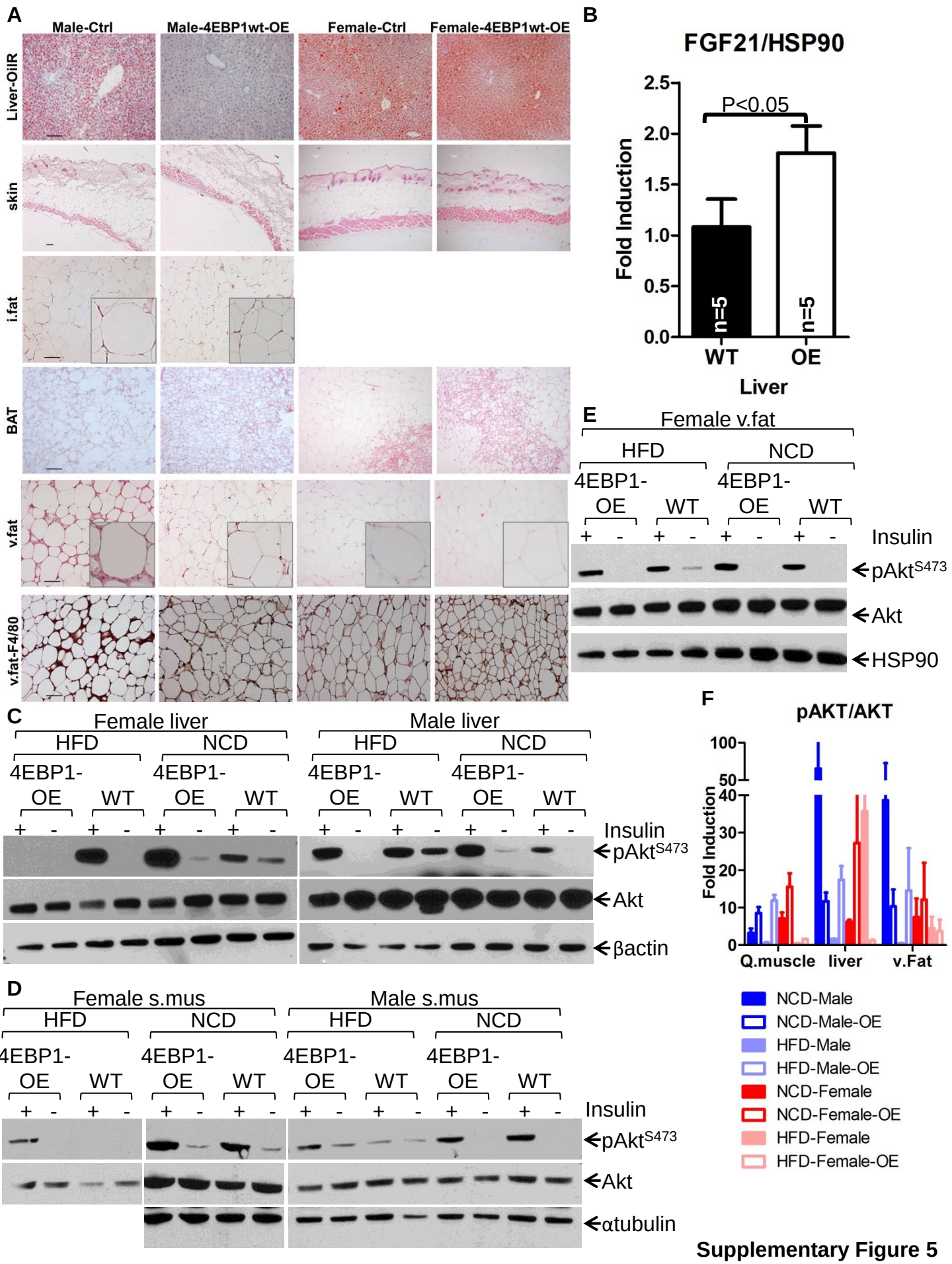
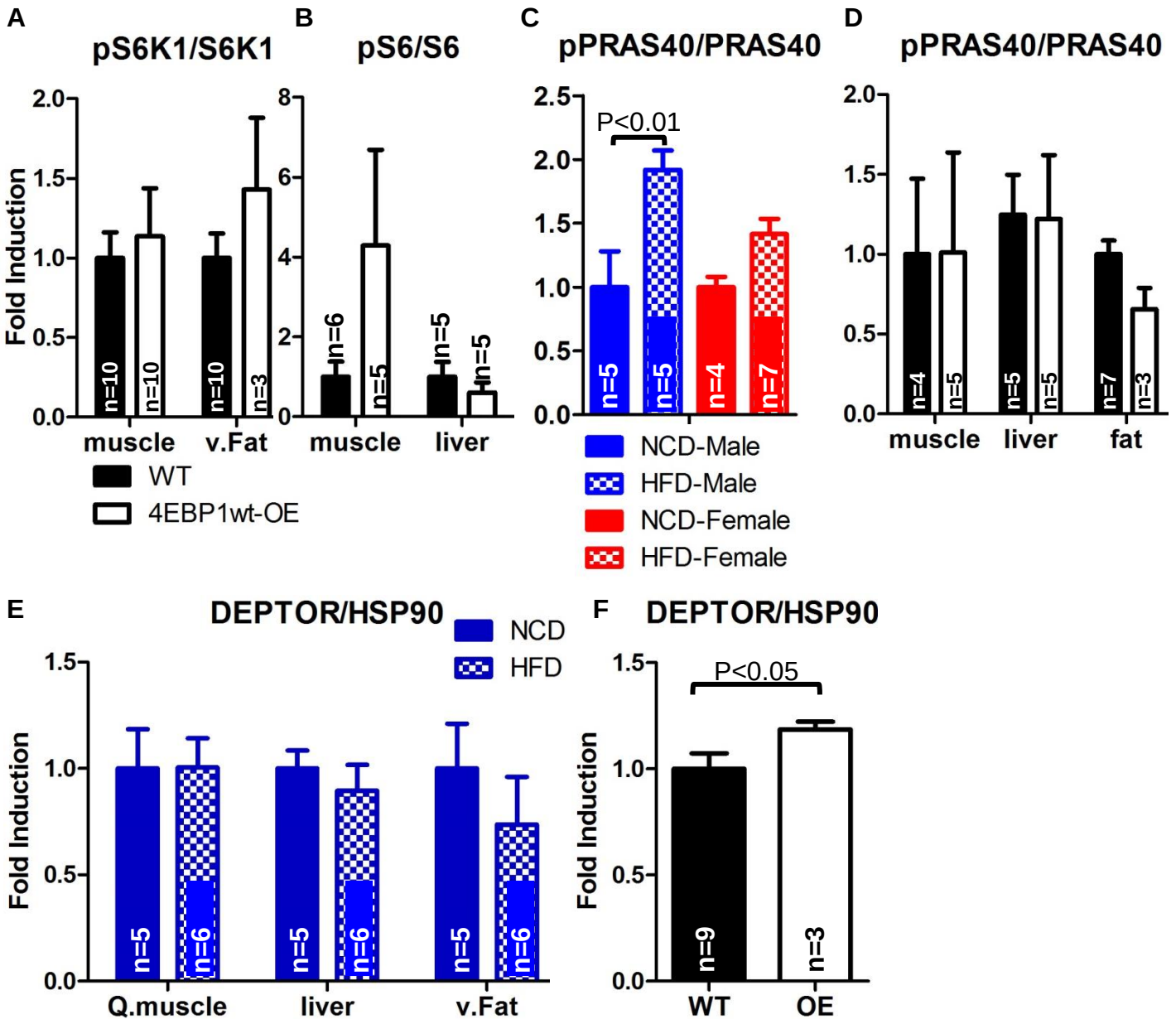
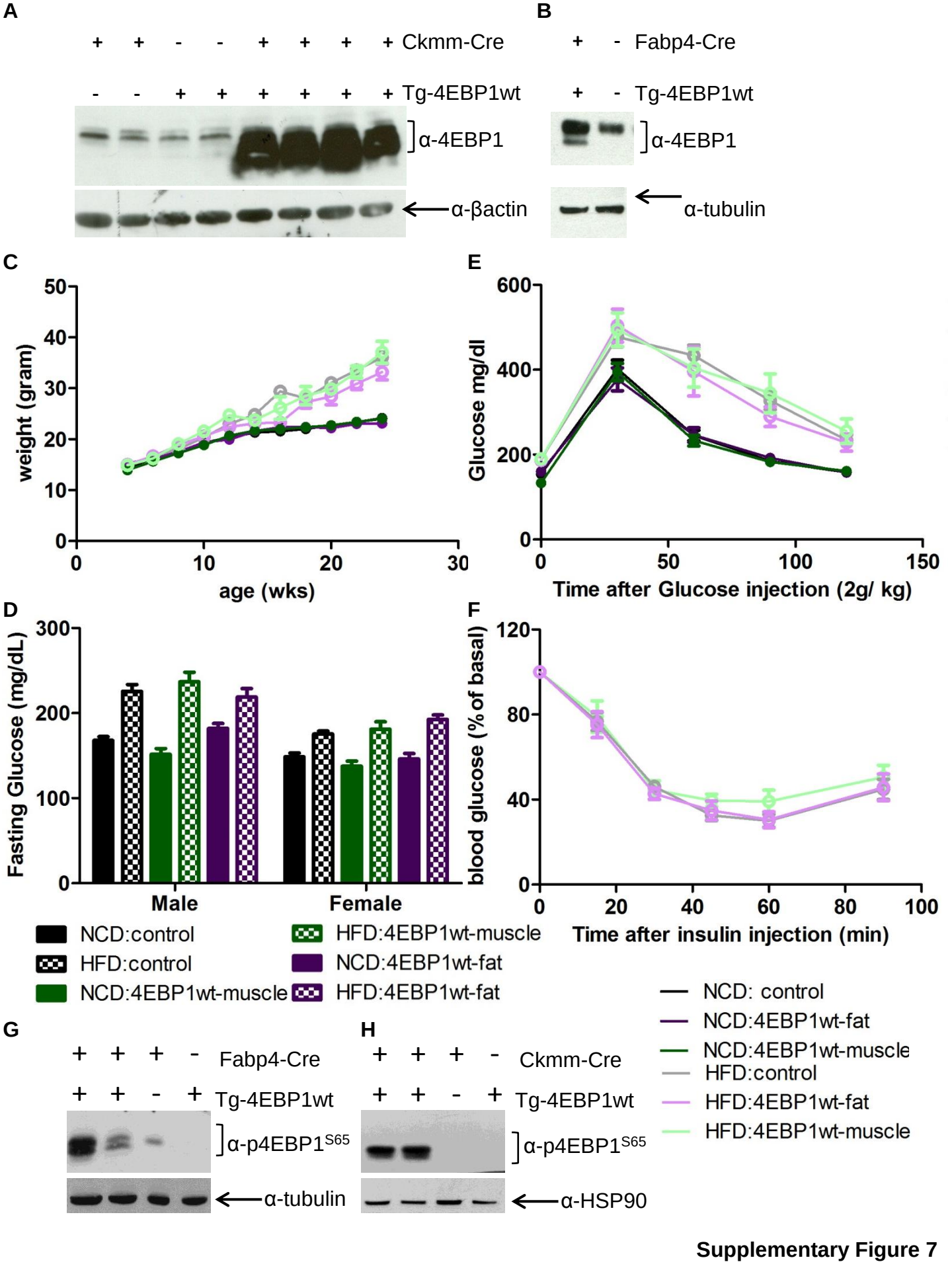


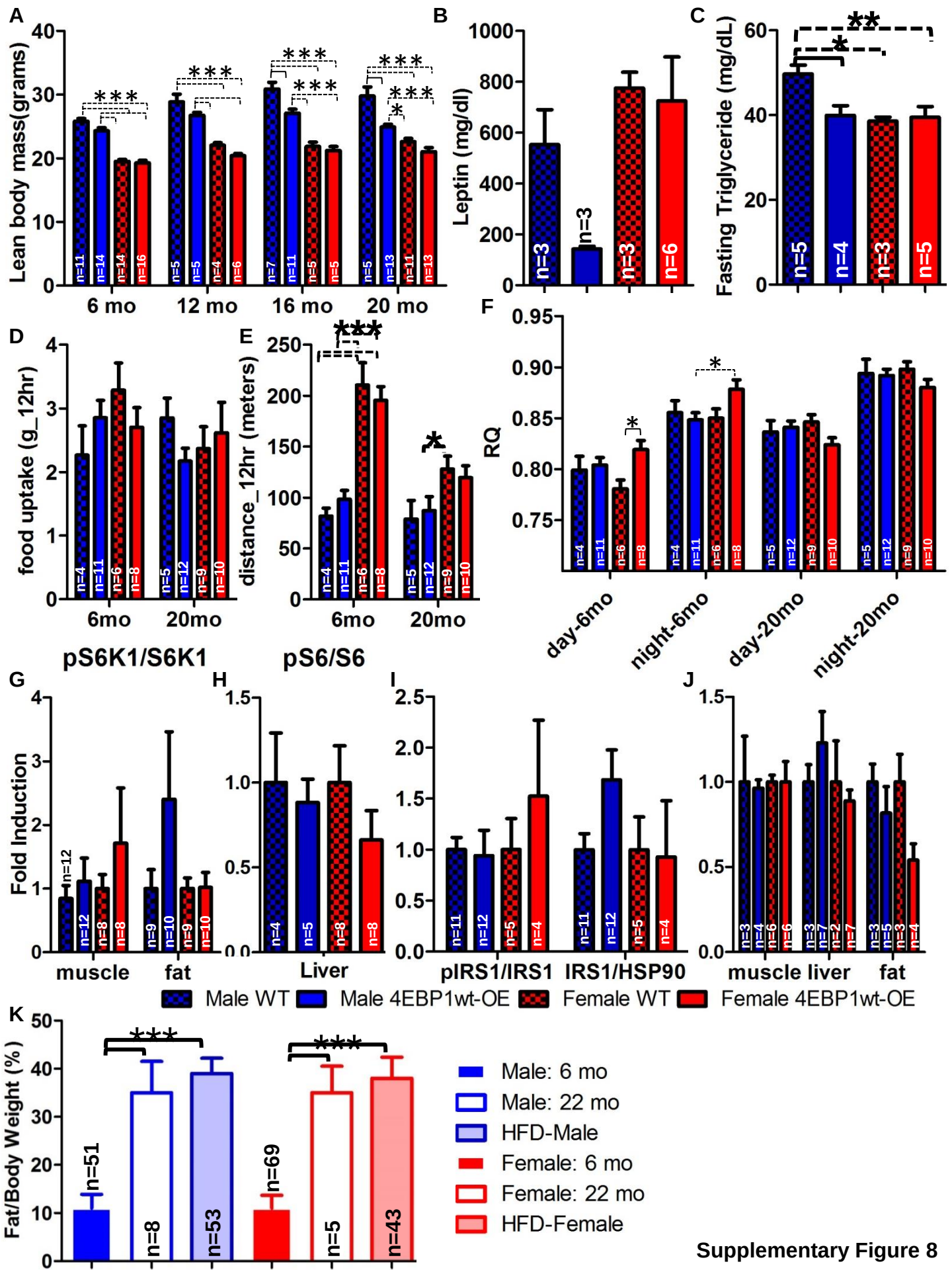
Supplementary Figure 5



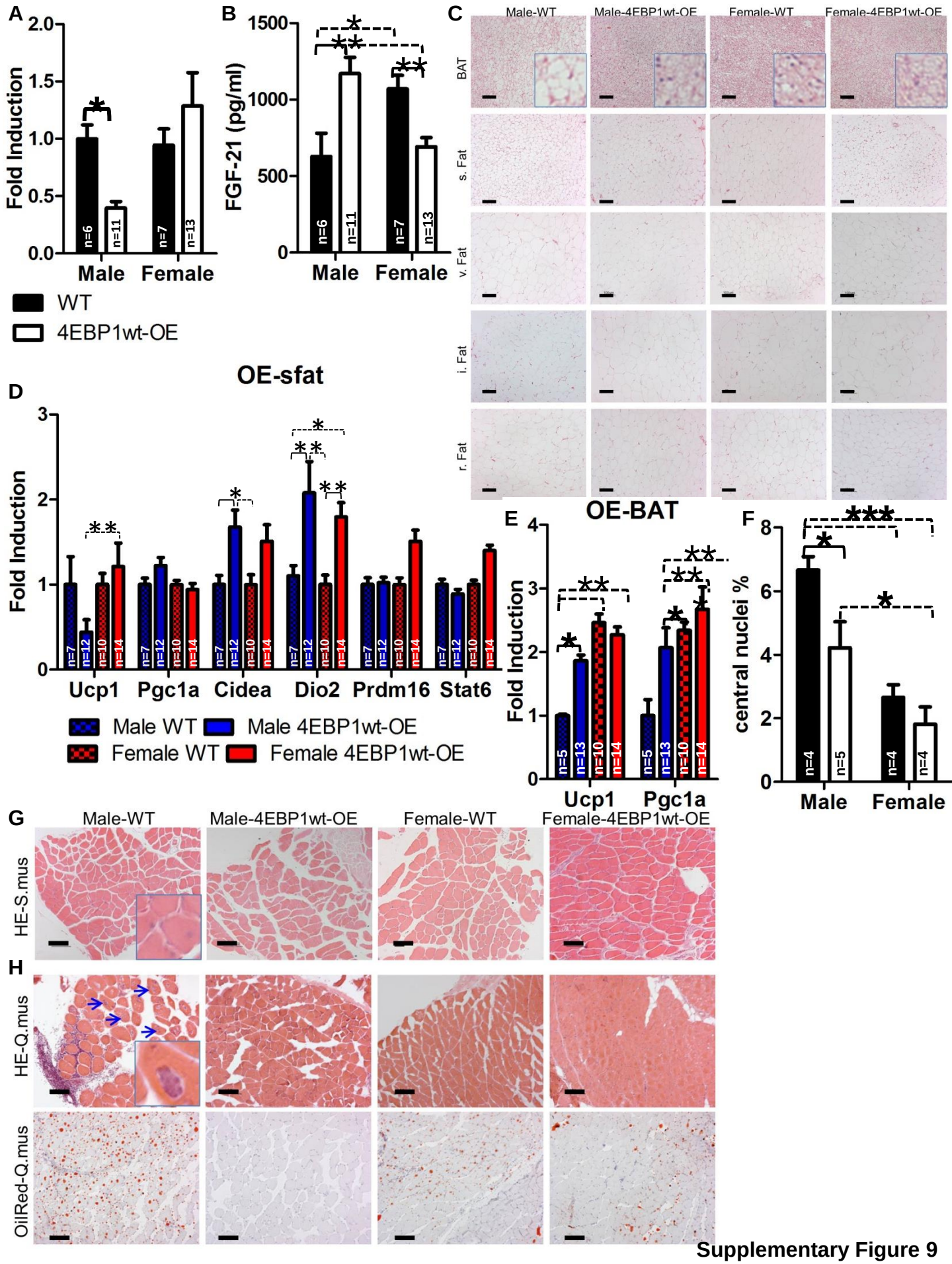
Supplementary Figure 6



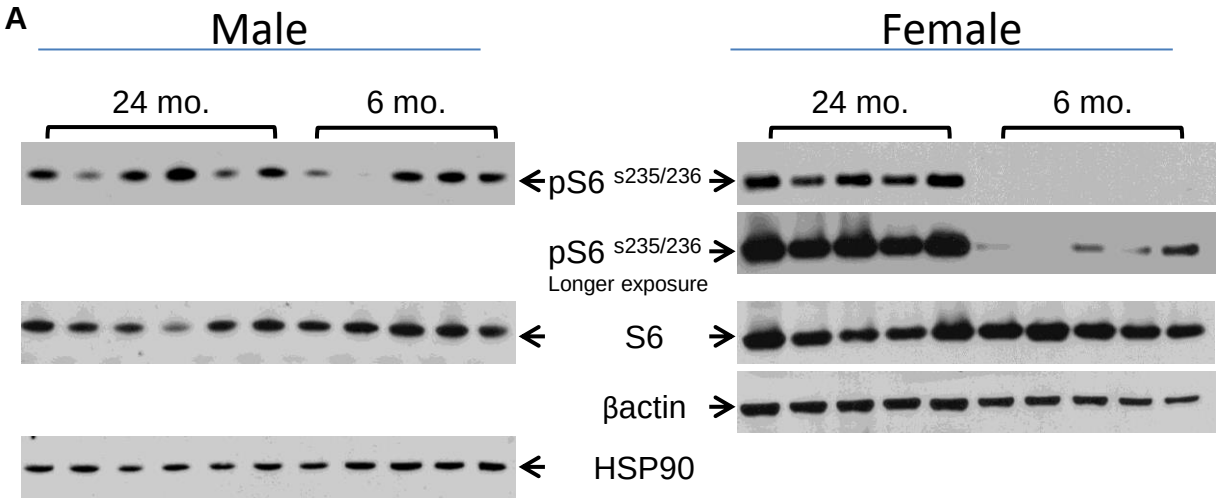
Supplementary Figure 7

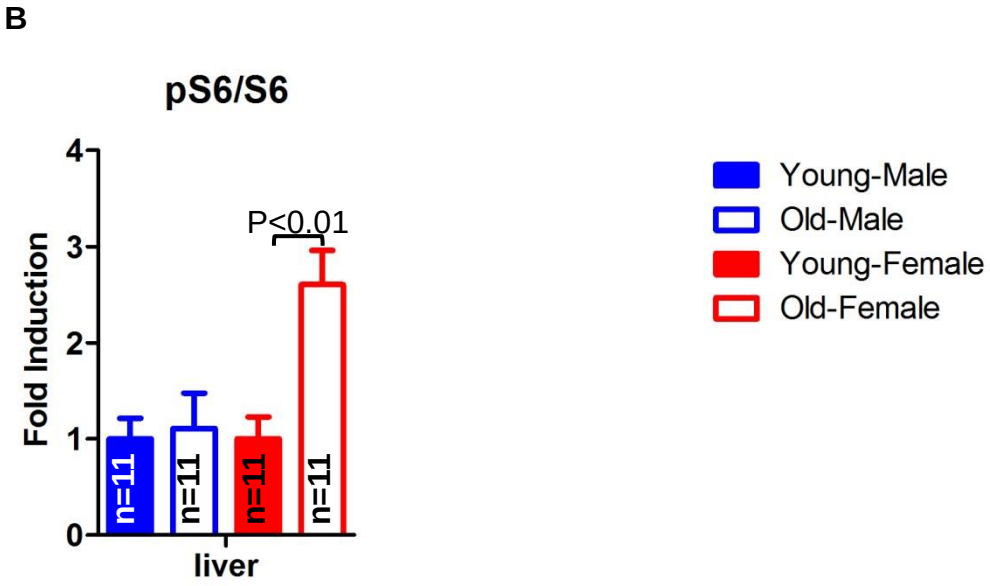


Supplementary Figure 8



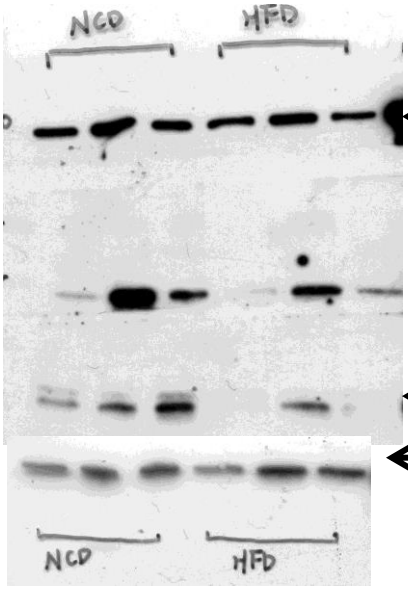
Supplementary Figure 9



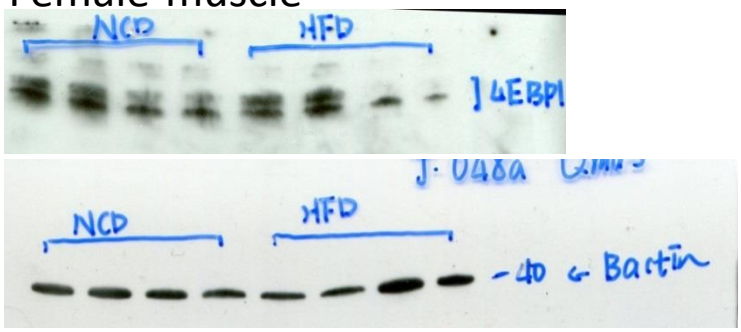


Supplementary Figure 10

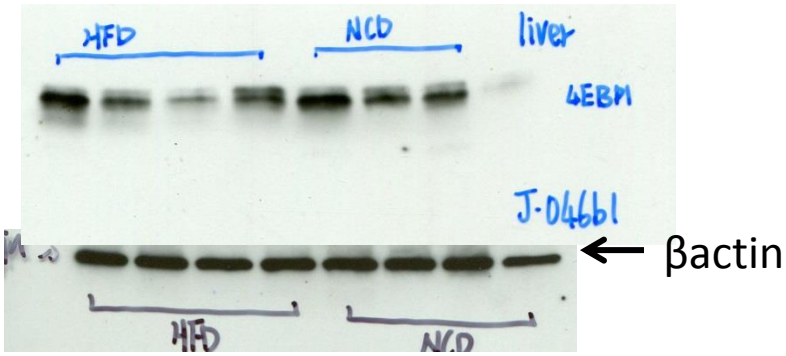
Male-muscle



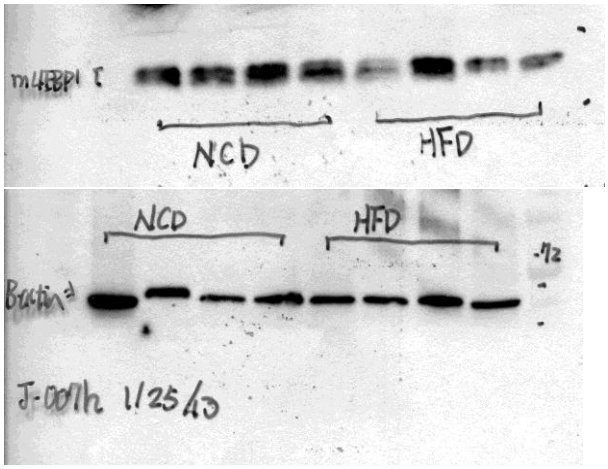
Female-muscle



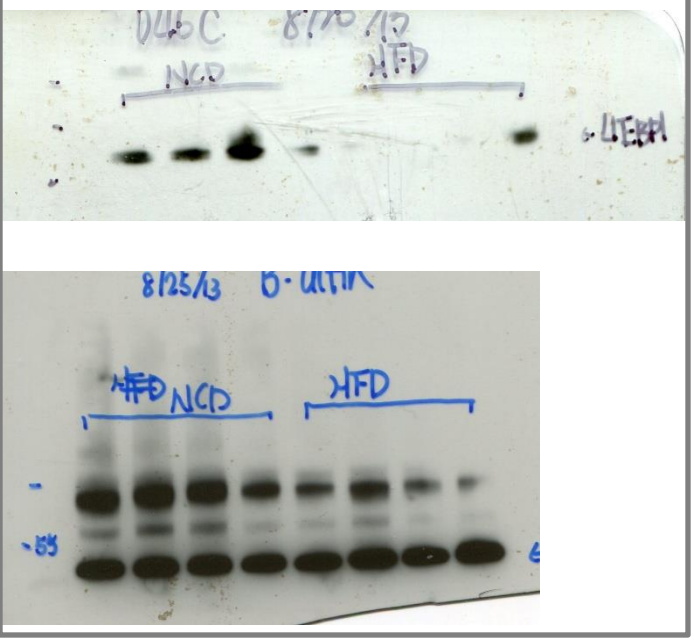
Male-liver



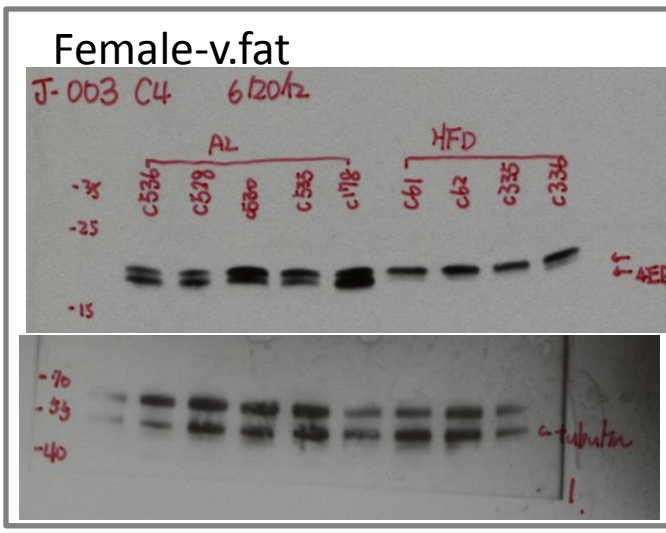
Female-liver



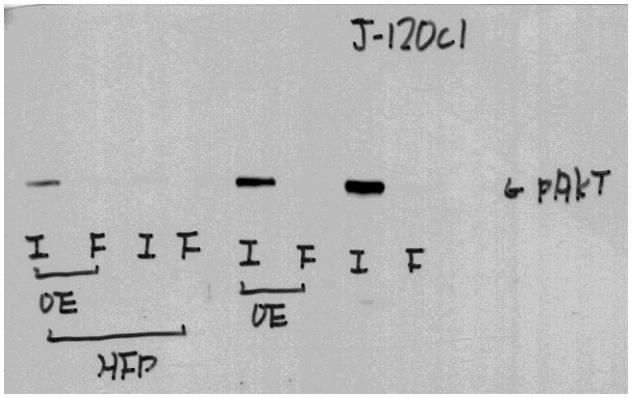
Male-v.fat

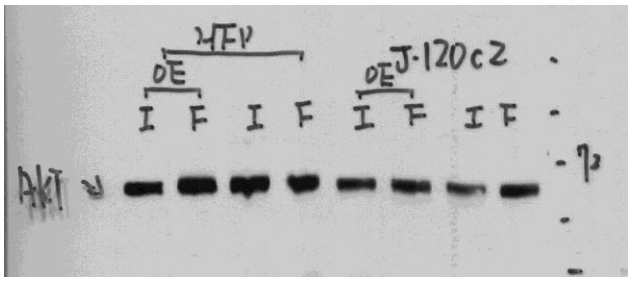


Female-v.fat



Uncropped scans- Fig 2





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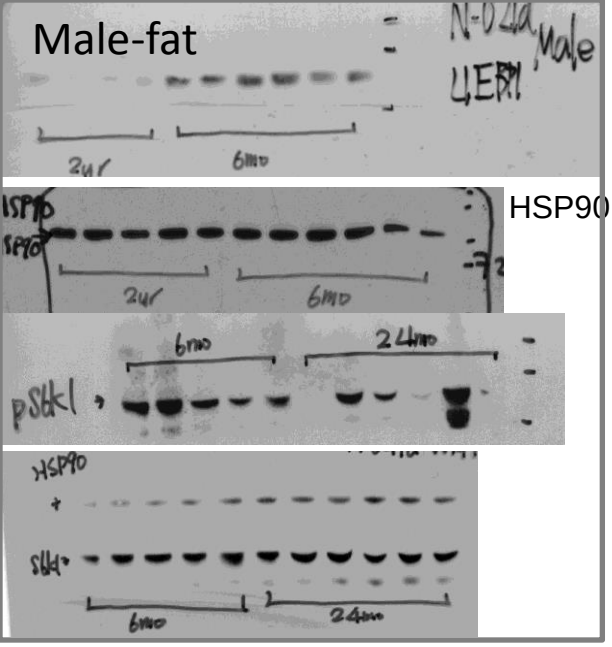
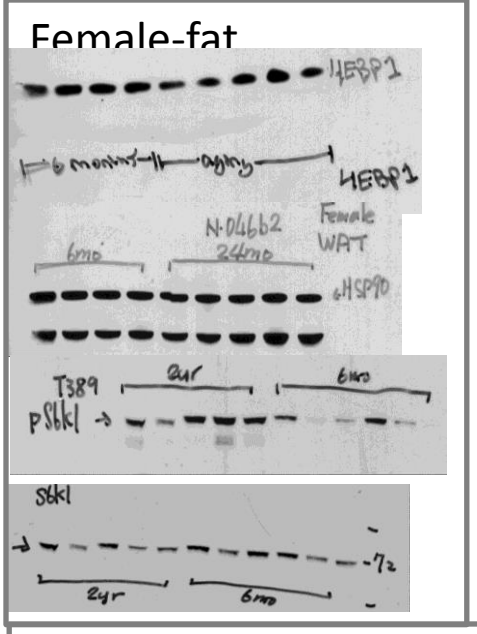
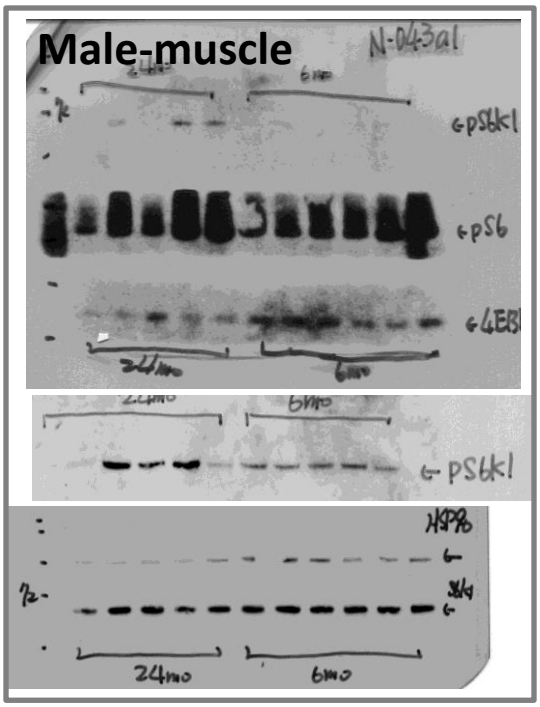
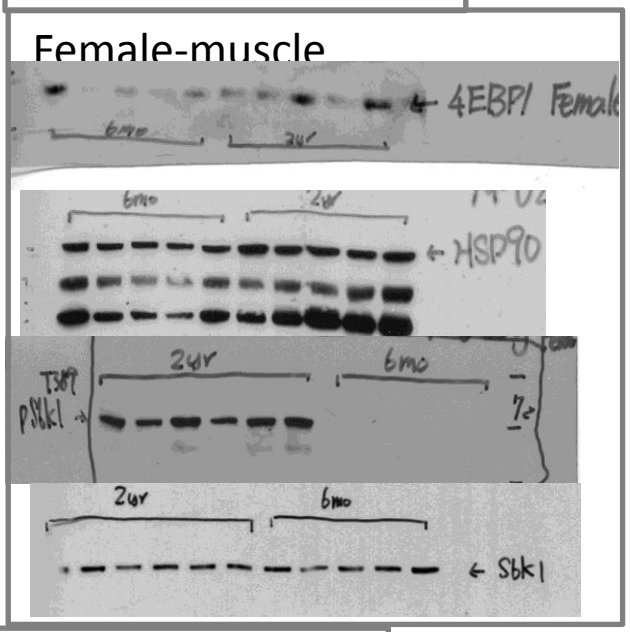
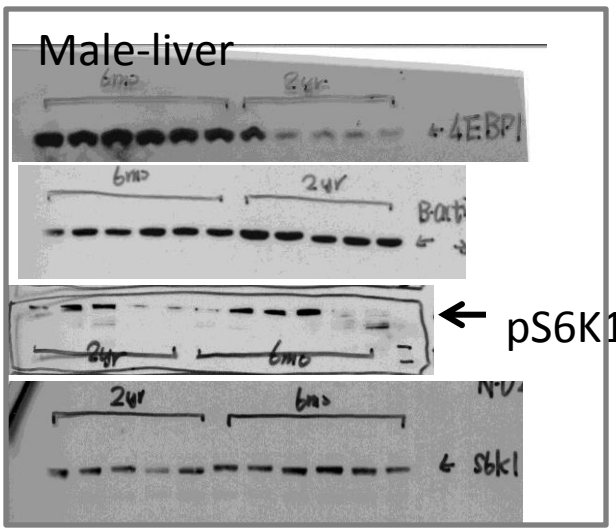
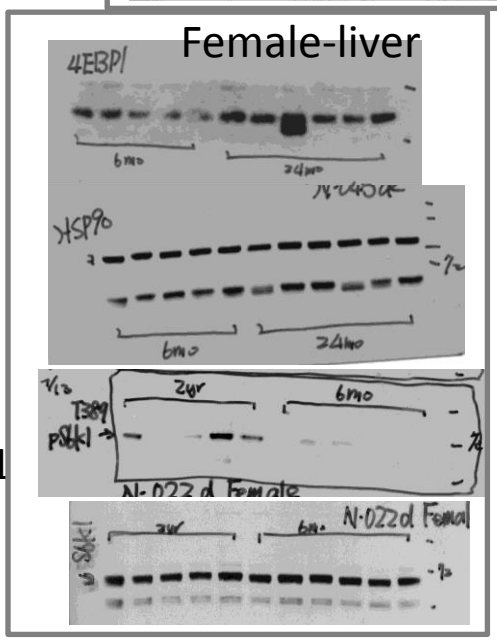






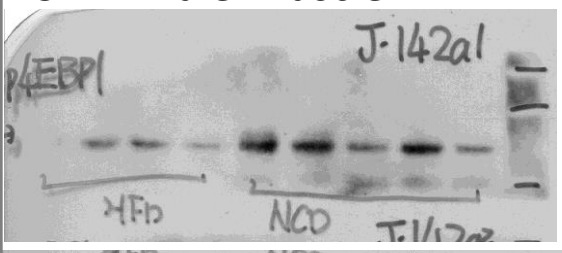


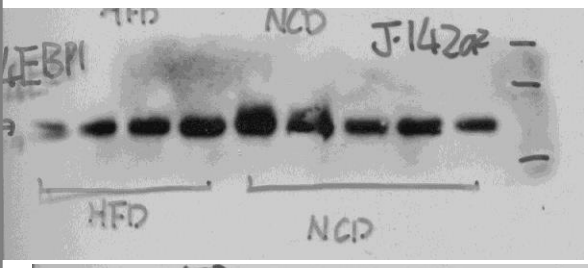


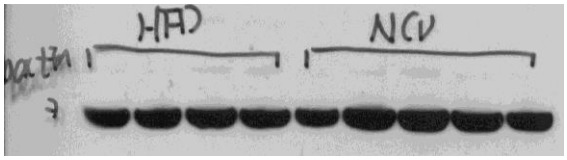


Uncropped scans- Fig 6

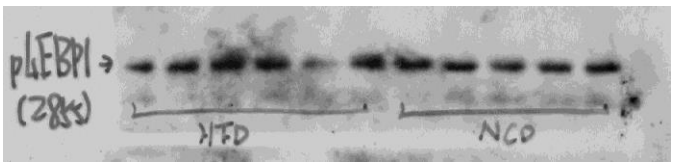
S2D: Male-muscle

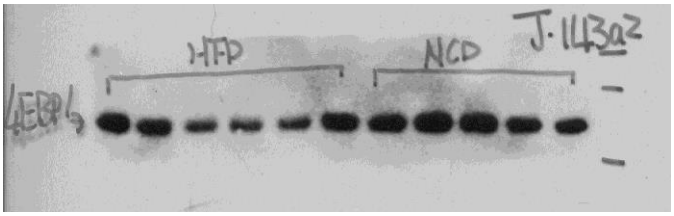


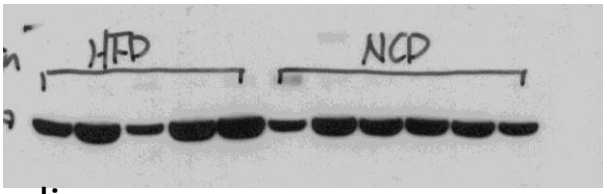




S2D: Female-muscle

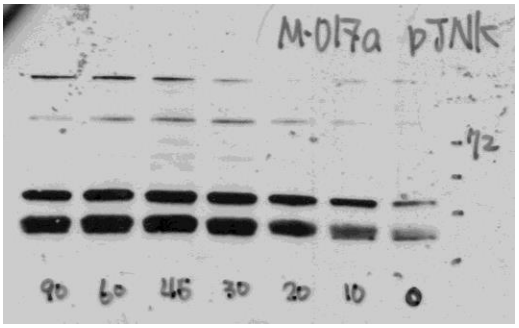


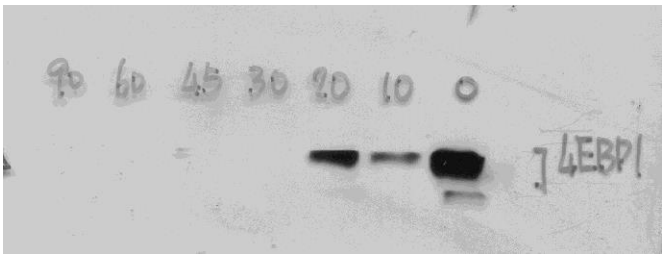


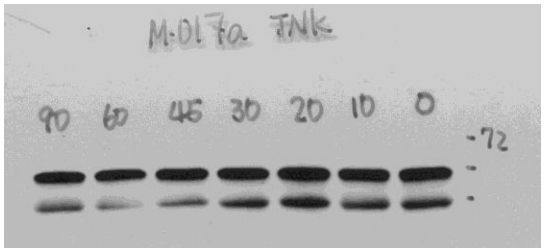


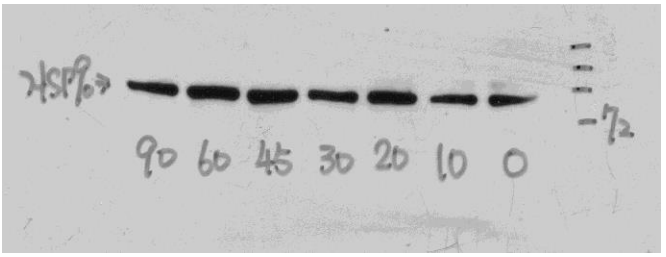
Tubulin

S2G



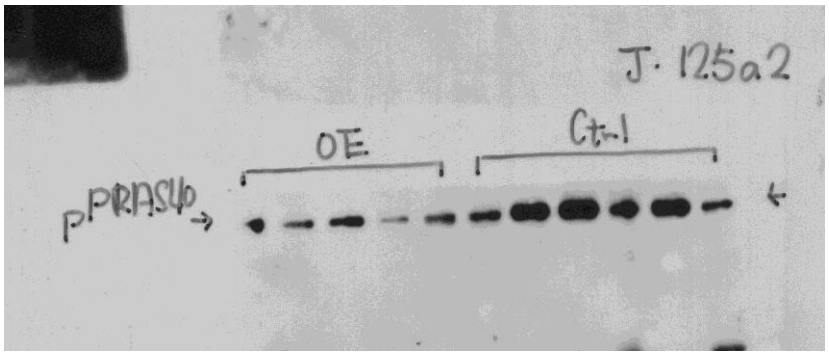


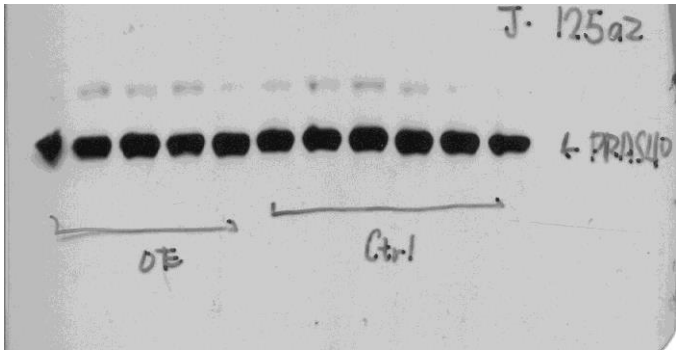




Uncropped scans- Fig S2

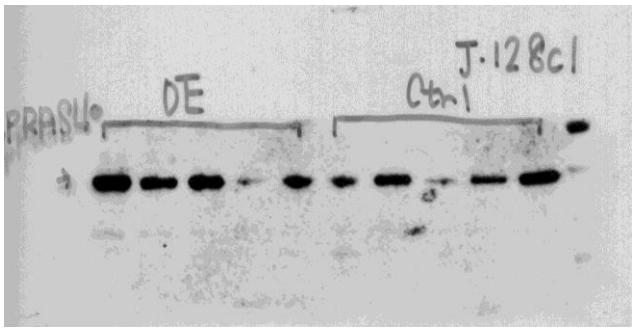
Male

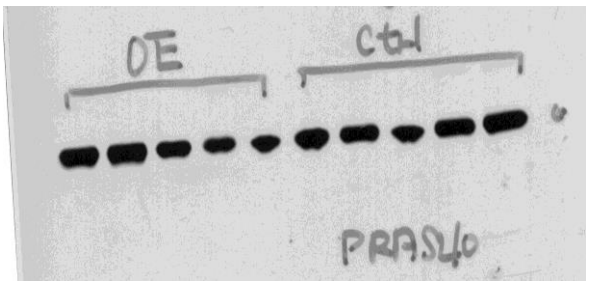




Female

pPRAS40





Uncropped scans- Fig S9A