Figure S1. Le Bacquer et al. 2006

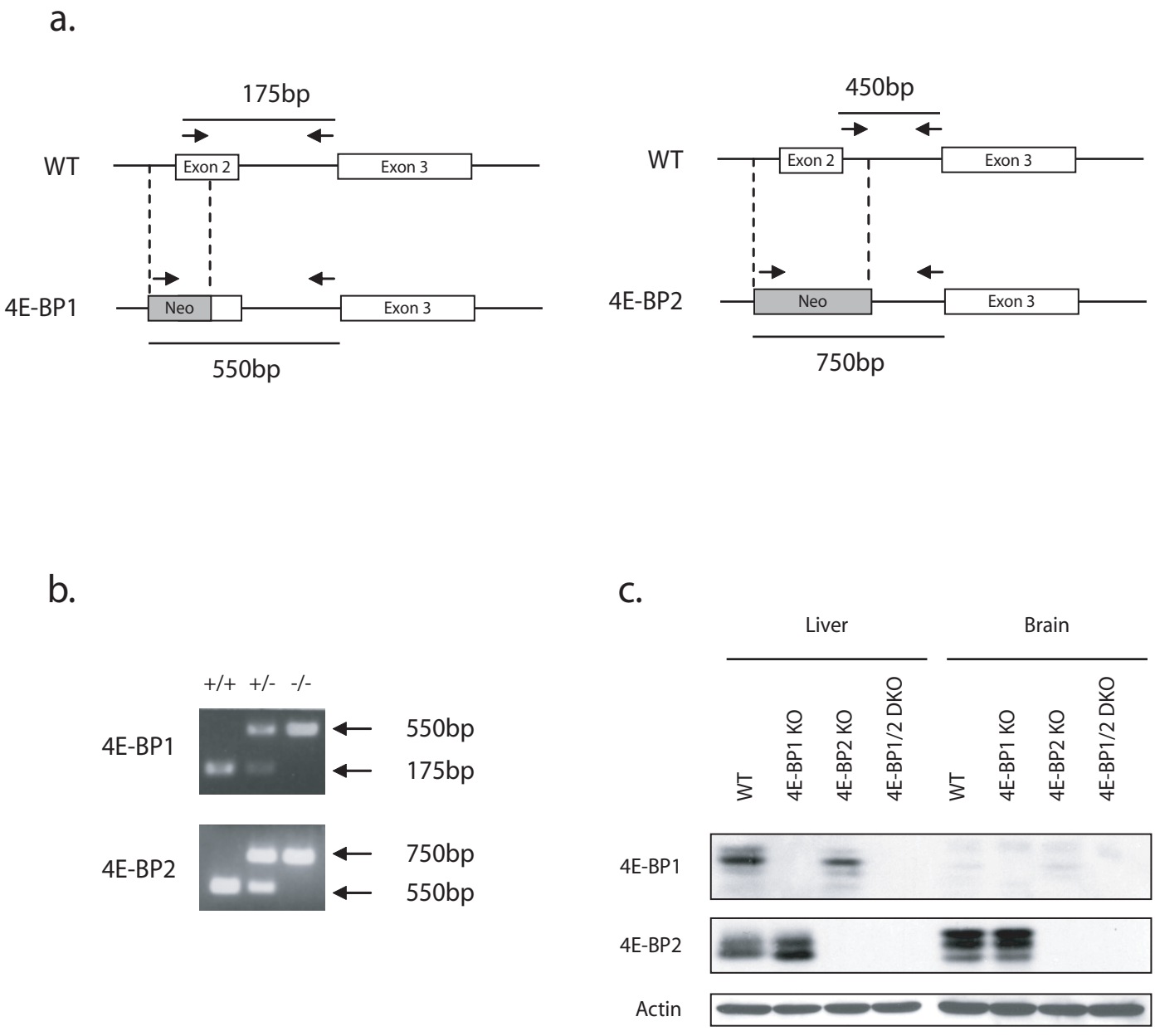


Figure S2. Le Bacquer et al. 2006

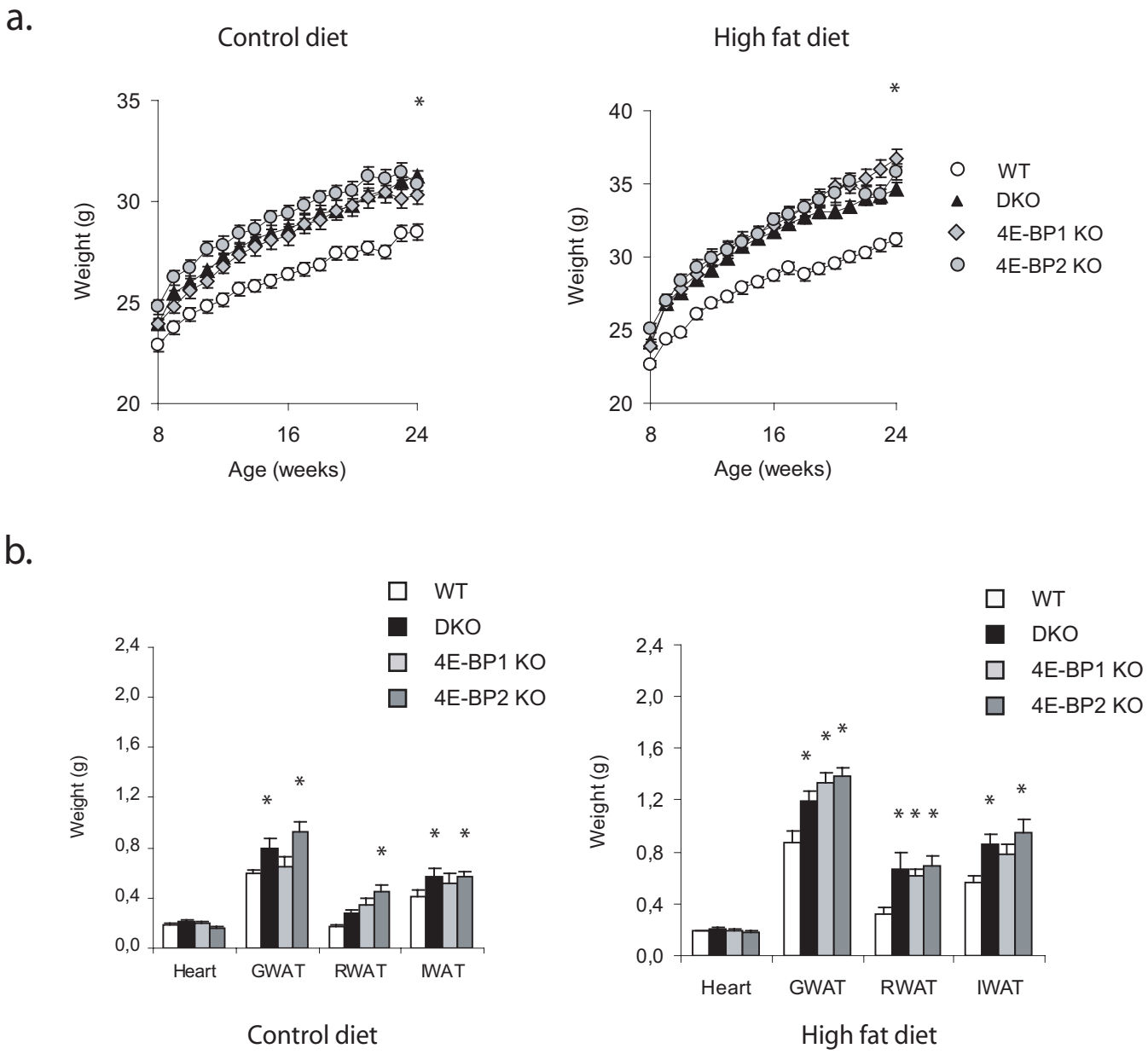


Figure S3. Le Bacquer et al. 2006

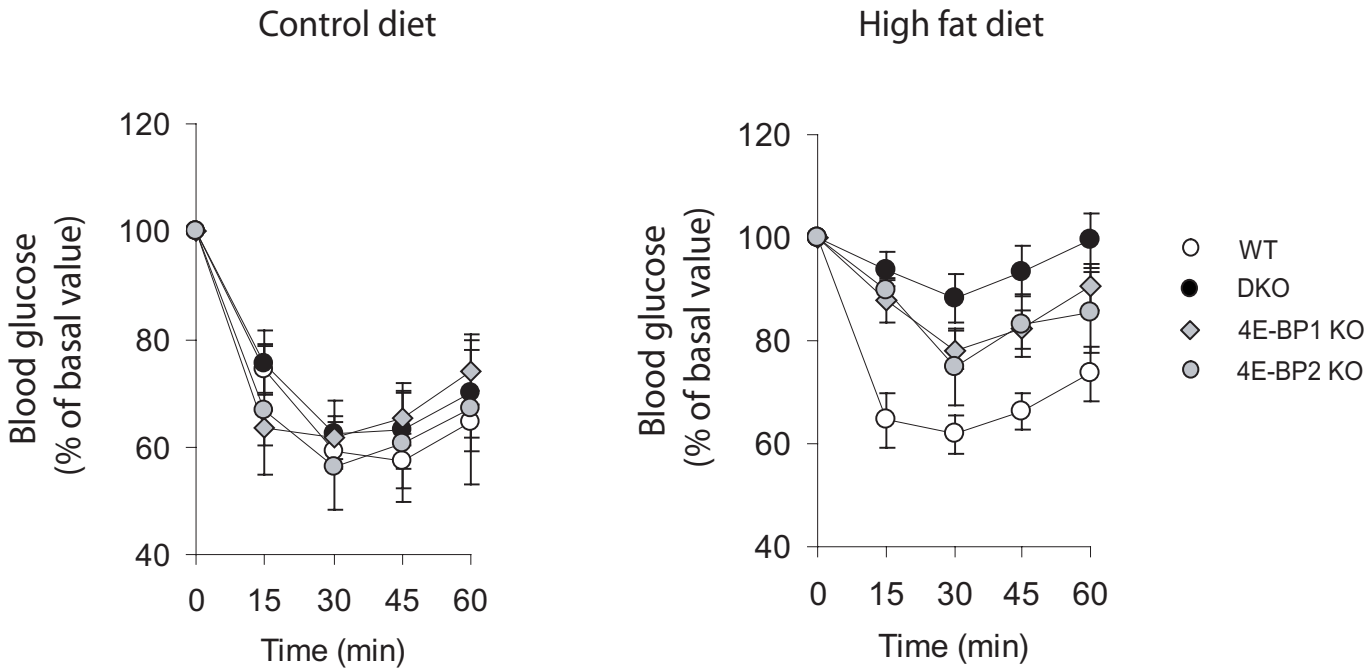
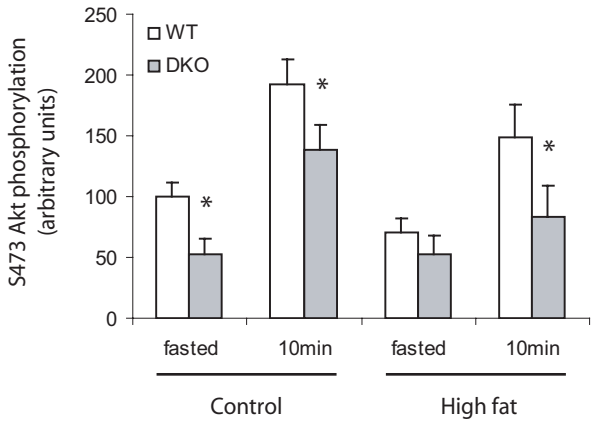
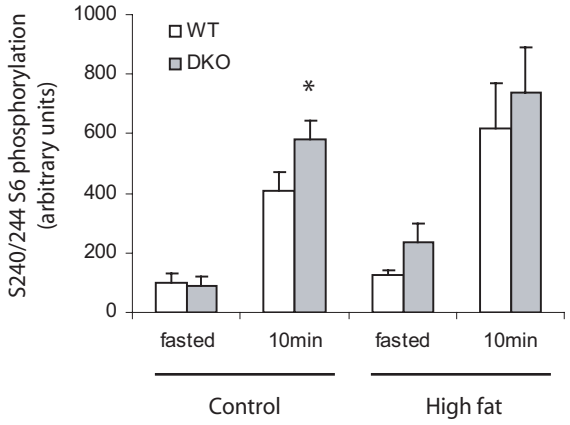
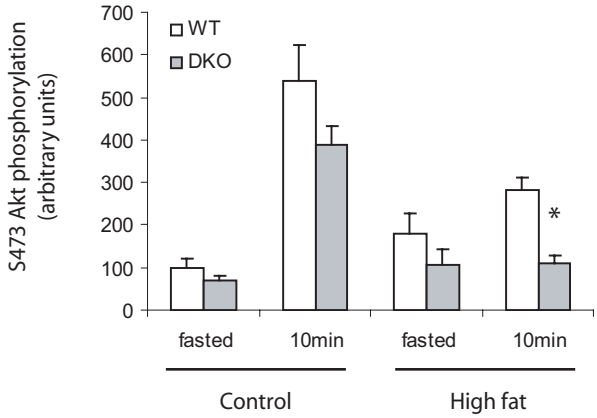


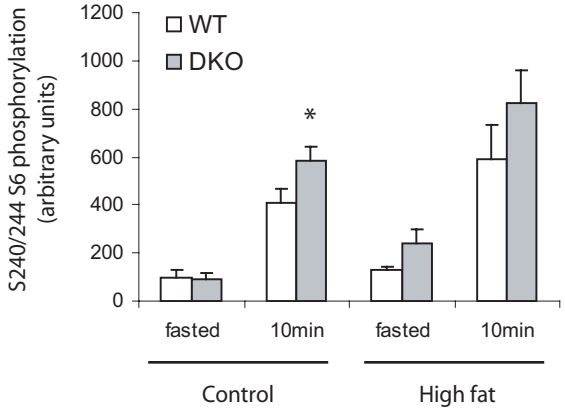
Figure S4. Le Bacquer et al. 2006



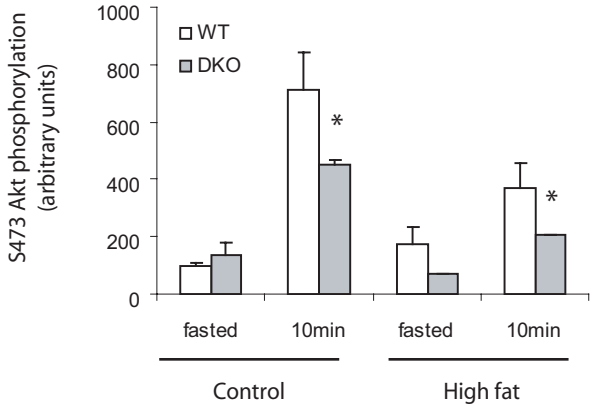


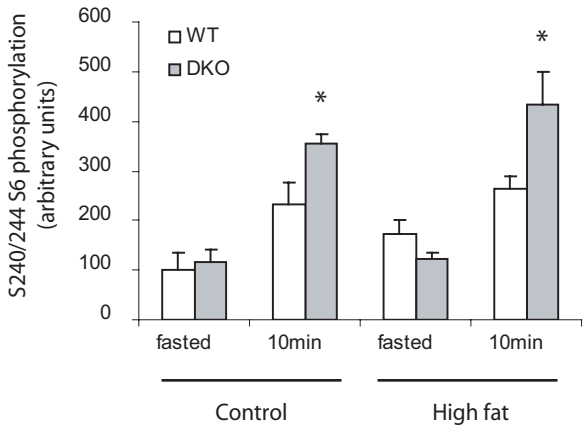
Adipose tissue





Liver

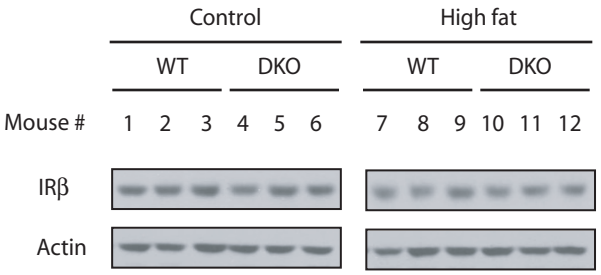




Muscle

Figure S5. Le Bacquer et al. 2006

a.



b.

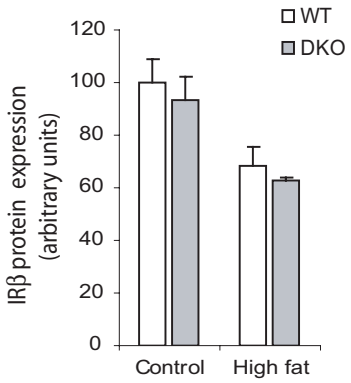
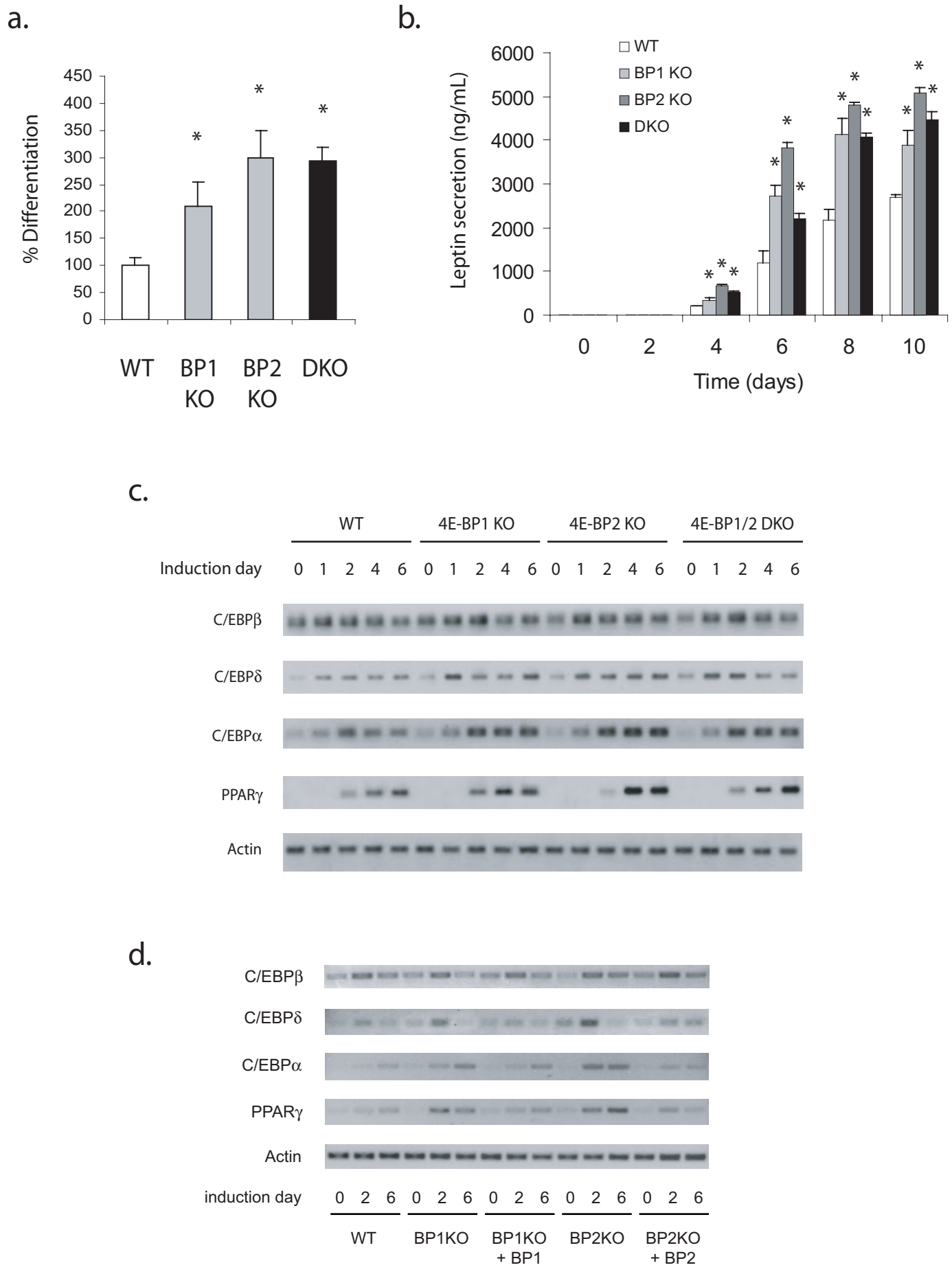


Figure S6. Le Bacquer et al. 2006



SUPPLEMENTAL RESULTS

Characterization of 4E-BP1 KO and 4E-BP2 KO mice. In a mixed genetic background (Balb/c + 129SvJ1), 4E-BP1 mutant mice are leaner than WT littermates (10). To determine whether the greater sensitivity to high fat diet-induced obesity exhibited by the DKO arose from the 4E-BP2 deletion, we fed 4E-BP1 KO and 4E-BP2 KO mice a control or high fat diet. Deletion of either 4E-BP1 or 4E-BP2 resulted in increased body weight as compared to WT; in addition, we **found no** significant difference in body weight between 4E-BP1 KO and 4E-BP2 KO mice on the control diet (Fig. S2). The difference in the body weight phenotype of Balb/c inbred *Eif4ebp1* and *Eif4ebp2* knockout mice and those in a mixed genetic background (10) is addressed in the Discussion. Blood parameters of 4E-BP1 KO, 4E-BP2 KO and WT mice were somewhat similar on the control diet; however, the HDL level was significantly increased in 4E-BP1 KO and 4E-BP2 KO mice, and cholesterol was significantly increased only in the 4E-BP2 KO as compared to WT (Table S1).

The high fat diet caused a greater increase in body weight gain in both the 4E-BP1 KO and 4E-BP2 KO mice (+24% and +23% respectively, $P<0.01$) as compared to WT mice. The high fat diet also resulted in increased insulin and HDL-cholesterol levels in 4E-BP1 KO and 4E-BP2 KO mice as compared to WT and led to increased cholesterol in 4E-BP2 KO mice (Table S1). Leptin levels were similar in 4E-BP single KO and WT mice, whereas they were significantly increased in DKO mice (Tables 1, S1). Despite high fat diet-associated adipose tissue enlargement and blood parameter perturbations observed in 4E-BP1 KO and 4E-BP2 KO mice (Table S1 and Fig. S2), the insulin

resistance was less pronounced in the 4E-BP single KOs as compared to DKO mice (Fig. S3). These data show that 4E-BP1 and 4E-BP2 may have a synergetic effect on the development of high fat diet-induced obesity and on the control of insulin sensitivity.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Disruption of 4E-BP1 and 4E-BP2 genes. (**a**) Schematic representation of mouse 4E-BP1 and 4E-BP2 gene targeting with the positions of the corresponding primers used for PCR-genotyping and sizes of the RT-PCR products indicated. (**b**) RT-PCR analysis of the wild-type (+/+), heterozygous (+/-) and knockout (-/-) 4E-BP1 and 4E-BP2 genotypes. For 4E-BP1 knockout genotyping, primers were as follows: The exon-2 sense primer (5'-GATGGAGTGTCGGAACTCACC-3'), the Neo-specific sense primer (5'-GCATCGAGCGAGCACGTACTC-3), and the intron 2-specific antisense primer (5'-GACCTGGACAGGACTCACCGC-3') were included in each RT-PCR reaction. For 4E-BP2 knockout genotyping, primers were as follows: The intron 2-specific sense primer (5'-GGTGGGACTGTCGGTCTTCTG-3'), the Neo-specific sense primer (5'-GCATCGAGCGAGCACGTACTC-3), and the intron 2-specific antisense primer (5'-CAGCACCTGGTCATAGCCGTG-3'). (**c**) Western blot analysis of 4E-BP1 and 4E-BP2 in wild-type (WT), 4E-BP1 KO, 4E-BP2 KO and DKO liver and brain tissue.

Figure S2. Increased obesity and insulin resistance in 4E-BP1 KO and 4E-BP2 KO mice. (**a**) Growth curve of WT, 4E-BP1 KO, 4E-BP2 KO, and DKO mice under normal chow

diet (control) or high fat diet. (**b**) Weight of heart, gonadal (GWAT), retroperitoneal (RWAT), and inguinal (IWAT) white adipose tissues. Values are means ± s.e.m. *, $p<0.05$.

Figure S3. Insulin resistance test in 24-week old WT, 4E-BP1 KO, 4E-BP2 KO, and 4E-BP1/2 DKO mice on control and high fat diets. Fed mice received an intraperitoneal injection of 0.75U/kg insulin. Insulin and blood samples were taken at the indicated times (n= 6-8) Values are means ± s.e.m. *, $p<0.05$

Figure S4. Ser473 Akt and ser240/244 S6 protein phosphorylation in WT and 4E-BP1/2 DKO mouse adipose, liver, and muscle tissues on control and high fat diets. Mice were fasted for 6h before receiving a 0.75U/kg insulin injection in the tail vein. Ten minutes later, the animals were sacrificed and tissues were collected for western blotting. Analysis was performed on 4 different experiments with ImageJ software (NIH).

Figure S5. Insulin receptor beta (IRβ) expression in WT and DKO adipose tissue. (**a**) Western blot analysis of IRβ. (**b**) Quantification of IRβ protein levels in WT and DKO adipose tissue. Levels were normalized to actin (n=6-7). Data are means ± s.e.m. Statistical analysis was performed with a two-tailed, unpaired, Student's *t*-test. *, $p<0.05$ compared with WT.

Figure S6. Increased adipogenesis in 4E-BP1 KO and 4E-BP2 KO MEFs. Primary MEFs from WT, 4E-BP1 KO and 4E-BP2 KO mice were grown to confluence and

differentiated into adipocytes as described in the Materials. (**a**) Quantification of lipid incorporation by measuring the intensity of Oil red O staining in WT, 4E-BP1 KO and 4E-BP2 KO MEFs at day 10 of the adipocyte differentiation process. (**b**) Quantification of leptin secretion. Secretion of leptin into the culture medium was measured throughout the adipogenesis induction period. (**c**) RT-PCR analysis showing C/EBPβ, C/EBPδ, C/EBPα and PPARγ expression following differentiation of WT, 4E-BP1 KO, and 4E-BP2 KO MEFs is shown. (**d**) RT-PCR analysis showing C/EBPβ, C/EBPδ, C/EBPα and PPARγexpression following differentiation of WT, single 4E-BP1 and 4E-BP2 KO MEFs and 4E-BP KO MEFs in which the corresponding missing 4E-BP was reintroduced is shown.

Table S1. Body weight and fasted insulin, glucose, cholesterol, triglycerides, and leptin levels of 4E-BP1 KO and 4E-BP2 KO mice on control and high fat diets.

	WT		4E-BP1/2 DKO		4E-BP1 KO		4E-BP2 KO	
	Control	High fat	Control	High fat	Control	High fat	Control	High fat
Weight (g)	28.5±0.4	31.2±0.6	31.3±0.2*	34.7±0.4*	30.3±0.5*	36.8±0.6*	30.8±0.3*	35.8±0.6*
Weight gain (g)	5.6±0.2	8.8±0.4	7.2±0.2*	10.8±0.4*	7.1±0.3*	9.8±0.5	6.1±0.4	8.4±0.4
Glucose (mg/dl)	87±8	146±7	72±5	180±10*	76±10	156±11	82±19	169±19
Insulin (mg/l)	1.92±0.29	2.36±0.49	2.51±0.30	4.40±0.71*	1.76±0.46	4.57±0.67*	1.50±0.20	3.57±0.27*
Cholesterol (mM)	4.20±0.17	4.69±0.19	5.02±0.22*	5.54±0.17*	4.36±0.22	5.12±0.14	4.87±0.17*	5.43±0.21*
HDL-Chol. (mM)	3.23±0.18	3.71±0.17	3.87±0.23	4.36±0.21*	3.69±0.21*	4.32±0.19*	4.43±0.13*	4.80±0.29*
NEFA (mM)	1.84±0.12	1.32±0.08	1.92±0.14	1.61±0.13	1.50±0.04	1.32±0.06	1.67±0.10	1.29±0.07
Triglycerides (mM)	2.05±0.16	1.20±0.06	2.12±0.12	1.44±0.10	1.46±0.09	1.20±0.06	1.85±0.09	1.40±0.04
Leptin (ng/ml)	12.6±1.4	21.3±3.8	16.1±2.0	37.4±4.8*	ND	31.3±2.5	ND	30.8±3.4

Mice were 24 weeks old. Data are presented as mean ± s.e.m. Statistical Analysis was performed with a two-tailed, unpaired, Student's *t*-test. *, $p < 0.05$ compared with wild-type (n = 10-15).