SUPPLEMENTAL DATA

SUPPLEMENTARY METHODS

***Grp78+/-* mouse genotyping.** For genotyping of the *Grp78* knockout allele, the primers PF3 (5'-GATTTGAACTCAGGACCTTCGGAAGAGCAG-3') and PTR (5'-TTGTTAGGGGTCGTTCACCTAGA-3') (1) were used in PCR analysis. Reactions were run under the following conditions: 94°C for 5 min, followed by 46 cycles of 94°C for 20 sec, 62°C for 30 sec, and 72°C for 30 sec, ended with 72°C for 7 min.

Hyperinsulinemic-euglycemic clamp. After 10-week HFD regimen, 20-week old *Grp78+/-* mice and their wild-type littermates were subjected to hyperinsulinemic-euglycemic clamp to assess insulin sensitivity *in vivo* as described (2). At 4~5 days before clamp experiments, mice were anesthetized, and an indwelling catheter was inserted in the right internal jugular vein (2). On the day of clamp experiment, a 3-way connector was attached to the catheter to intravenously deliver solutions (e.g., glucose, insulin). Mice were placed in a rat-size restrainer (to minimize stress during experiments in awake state) and tail-restrained using a tape to obtain blood samples from the tail vessels.

Following overnight fast (~15 hr), a 2 hr hyperinsulinemic-euglycemic clamp was conducted in awake mice with a primed (150 mU/kg body weight) and continuous infusion of human regular insulin (Humulin; Eli Lilly, Indianapolis, IN) at a rate of 2.5 mU/kg/min to raise plasma insulin within a physiological range (2). Blood samples (20 μl) were collected at 20 min intervals for the immediate measurement of plasma glucose concentration, and 20% glucose was infused at variable rates to maintain glucose at basal concentrations. Basal and insulin-stimulated whole body glucose turnover were estimated with a continuous infusion of [3-^{3}H] glucose (Perkin Elmer, Boston, MA) for 2 hr prior to the clamps (0.05 μCi/min) and throughout the clamps (0.1 μCi/min), respectively. All infusions were performed using microdialysis pumps (CMA/Microdialysis, North Chelmsford, MA). To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-D-[1-^{14}C]glucose (2-[^{14}C]DG) was administered as a bolus (10 μCi) at 75 min after the start of clamps. Blood samples were taken before, during, and at the end of clamps for the measurement of plasma [^{3}H]glucose, $^{3}H_2O$, 2-[^{14}C]DG concentrations, and/or insulin concentrations. At the end of the clamps, mice were euthanized, and tissues were taken for biochemical and molecular analysis (2).

Biochemical assays. Glucose concentrations during clamps were analyzed using 10 μl plasma by a glucose oxidase method on a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA). Plasma insulin concentrations were measured by ELISA using kits from Alpco Diagnostics (Salem, NH). Plasma triglyceride and FFA concentrations were measured using Sigma diagnostic kits (Sigma Diagnostics, St Louis, MO) and spectrophotometry. Plasma concentrations of [3-^{3}H]glucose, 2-[^{14}C]DG, and $^{3}H_2O$ were determined following deproteinization of plasma samples as previously described (2). The radioactivity of ^{3}H in tissue glycogen was determined by digesting tissue samples in KOH and precipitating glycogen with ethanol. For the determination of tissue 2-[^{14}C]DG-6-P content, tissue samples were homogenized, and the supernatants were subjected to an ion-exchange column to separate 2-[^{14}C]DG-6-P from 2-[^{14}C]DG..

Calculations. Rates of basal hepatic glucose production (HGP) and insulin-stimulated whole body glucose turnover were determined as the ratio of the [^{3}H]glucose infusion rate to the

specific activity of plasma glucose at the end of the basal period and during the final 30 min of clamp, respectively (2). Insulin-stimulated rate of HGP during clamp was determined by subtracting the glucose infusion rate from whole body glucose turnover. Whole body glycolysis and glycogen plus lipid synthesis from glucose were calculated as previously described (2). Insulin-stimulated glucose uptake in individual tissues was assessed by determining the tissue content of 2-[^{14}C]DG-6-P and plasma 2-[^{14}C]DG profile.

RT-PCR. Tissue samples from individual mice were homogenized and extracted for RNA using TRI reagent (Sigma). cDNA samples were prepared using SuperScript™ II Reverse Transcriptase (Invitrogen). To assess mRNA levels of specific genes, the following primer pairs were applied: Mb-actin1 (5'-GACGGCCAGGTCATCACTAT-3') and Mb-actin2 (5'-GTACTTGCGCTCAGGAGGAG-3') for *β-actin* (3); mG78ex5F (5'-GCAGAAACTCCGGCGTGAGG-3') and mG78ex6R (5'-GGCTCCTTGCCATTGAAGAAC-3') for *Grp78*; mG94ex16F (5'-ACGGCAACACTTCGGTCAGG-3') and mG94ex18R (5'-CCACACGGGATTCATAGCGAG-3') for *Grp94*; mPDIex2F (5'-GCCCCTGAGTATGAAGCTGC-3') and mPDIex5R (5'-TCTCTCAAGTTGCTGGCTGC-3') for *PDI*; mCNXex8F (5'-GGCCCAAAATAGCAGATCCAG-3') and mCNXex12R (5'-GCCTCCAGCATCTGCAGCAC-3') for *calnexin (CNX)*; mCRTex1F (5'-CCGCTGCCTGAAGATCGTC-3') and mCRTex3R (5'-TGTCCTTCTGGTCCAAACCAC-3') for *calreticulin (CRT)*.

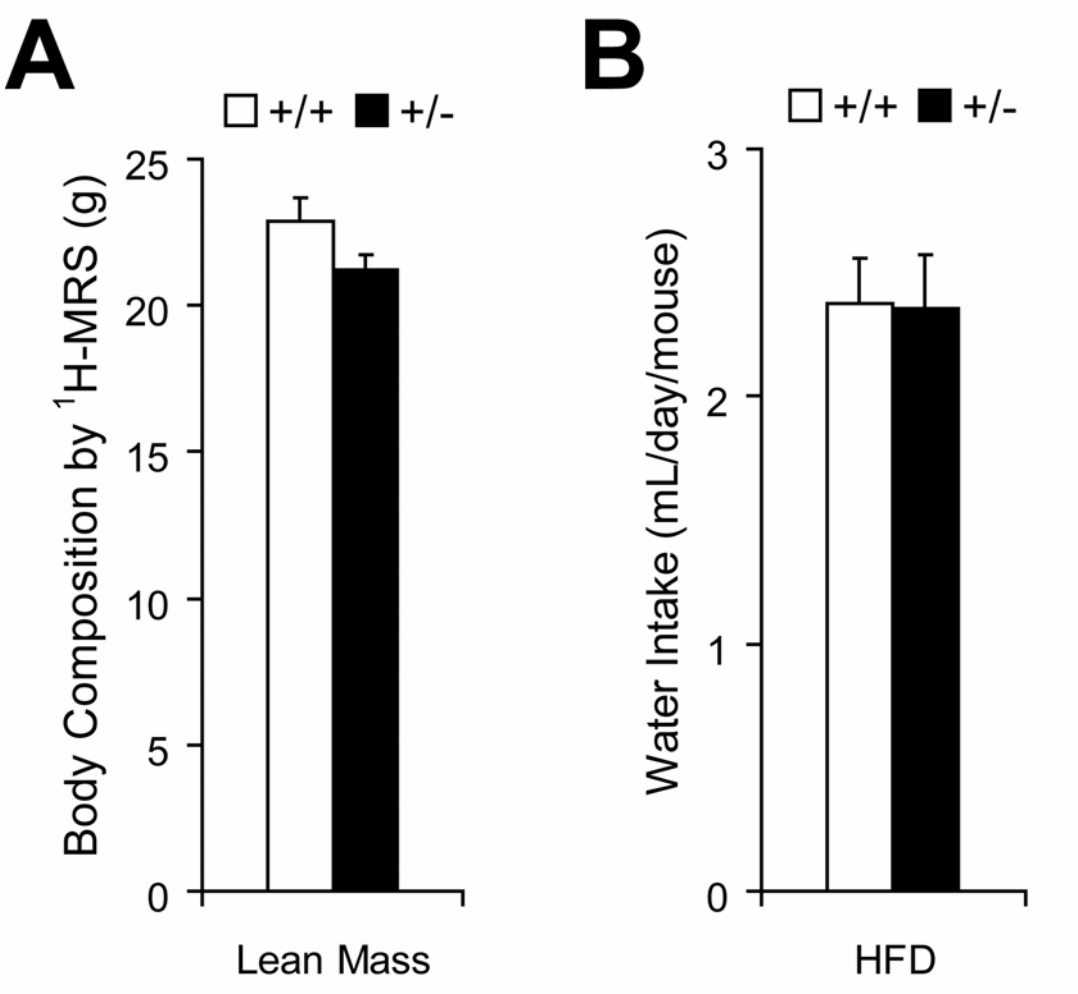
Immunohistochemistry. Paraffin sections of formalin-fixed pancreas were stained with glucagon (1:100, Signet).

References

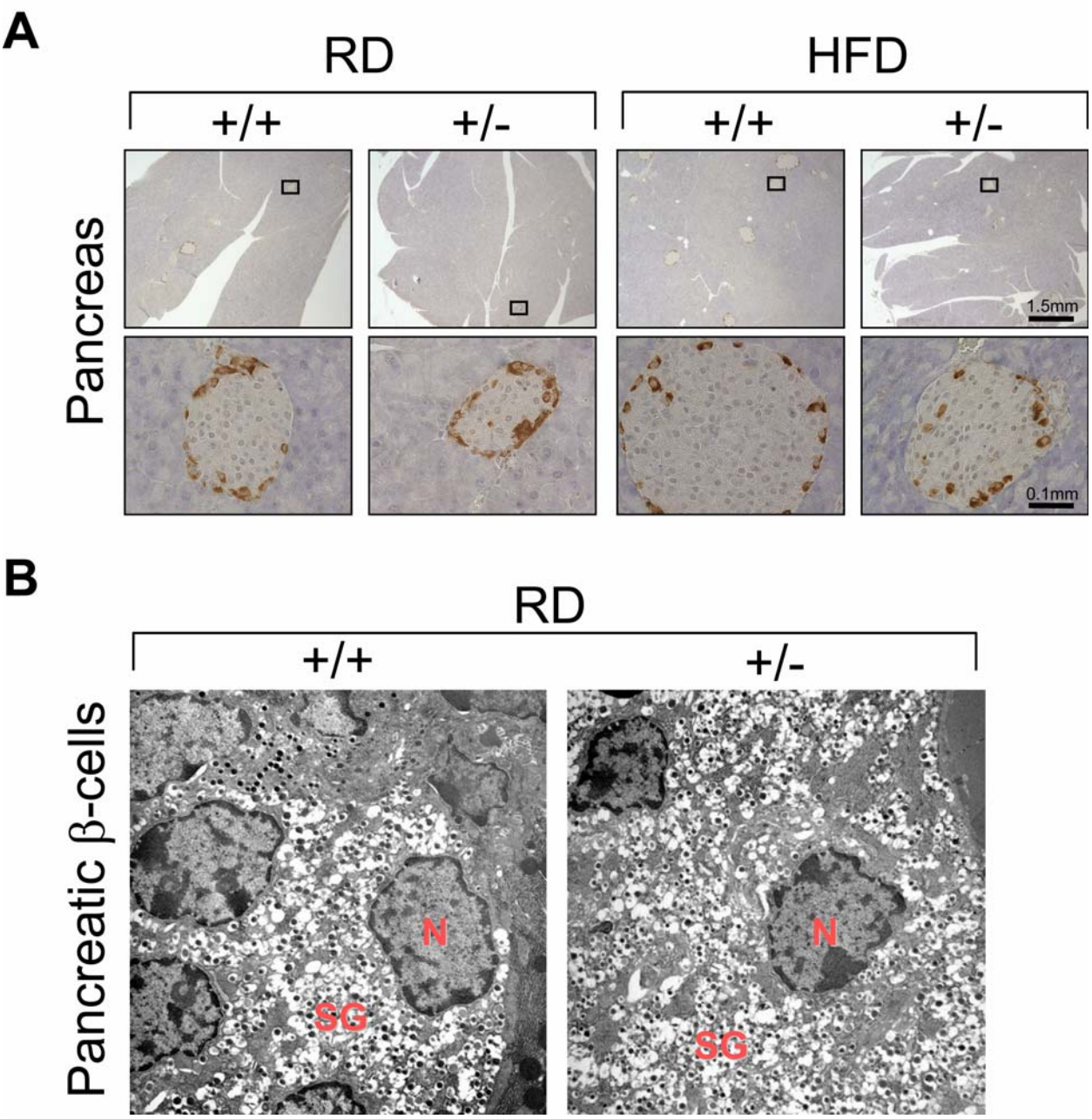
1. Luo S, Mao C, Lee B, Lee AS. GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. Mol Cell Biol 2006;26:5688-5697
2. Kim HJ, Higashimori T, Park SY, Choi H, Dong J, Kim YJ, Noh HL, Cho YR, Cline G, Kim YB, Kim JK. Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action *in vivo*. Diabetes 2004;53:1060-1067
3. Ni M, Zhou H, Wey S, Baumeister P, Lee AS. Regulation of PERK signaling by a novel cytosolic isoform of the UPR regulator GRP78/BiP. PLoS ONE, in press.

SUPPLEMENTARY FIGURES

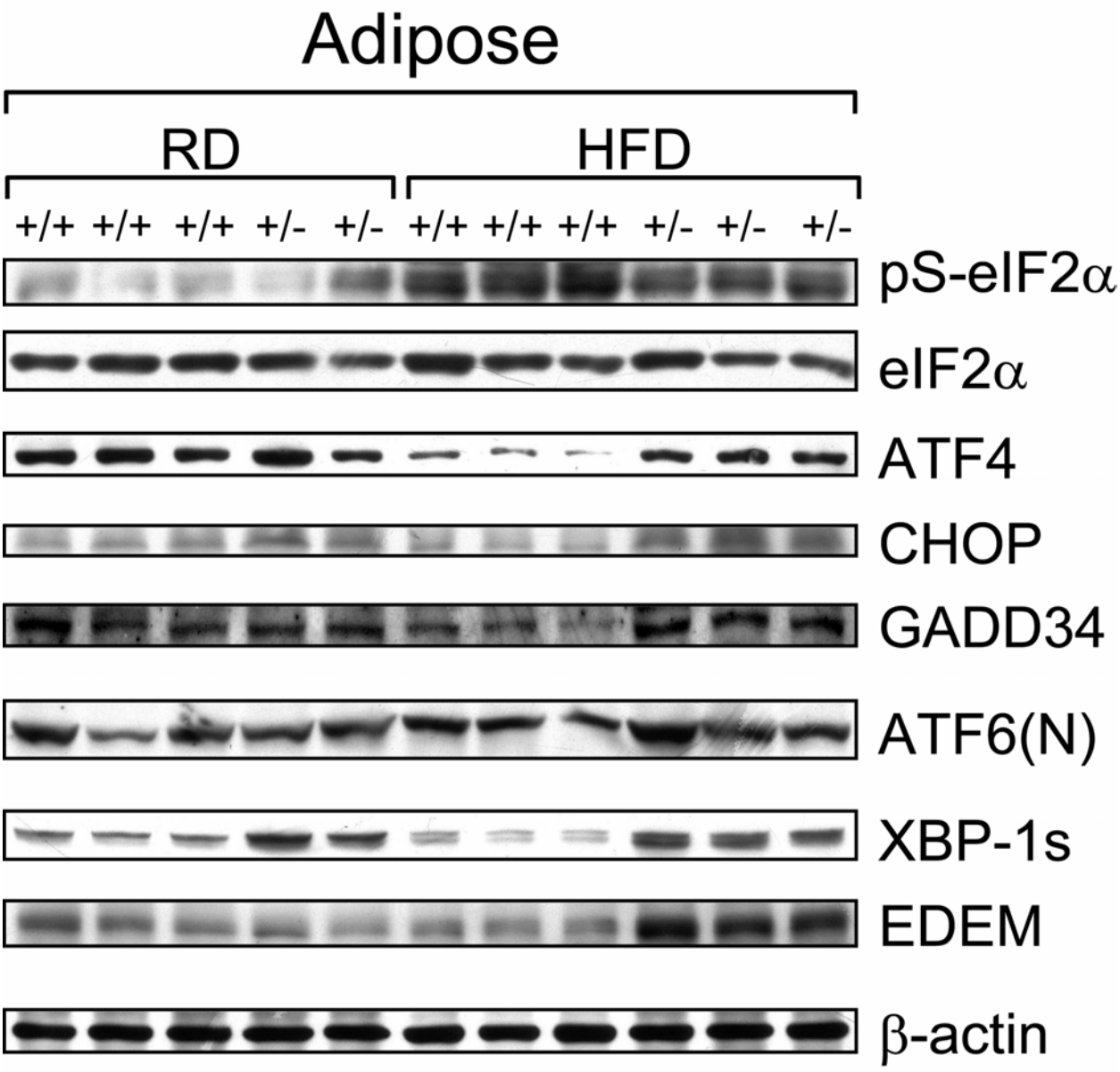
Supplementary Figure 1. Body composition and metabolic phenotyping of *Grp78+/-* mice on HFD. *A*: Lean mass of *Grp78+/-* (n=6) and +/+ (n=9) mice after 10-week HFD, analyzed by ^{1}H-MRS. *B*: Water intake of *Grp78+/-* (n=7) and +/+ (n=5) mice after 10-week HFD, measured in metabolic cages for 3 successive days. Data are all expressed as mean±SEM.



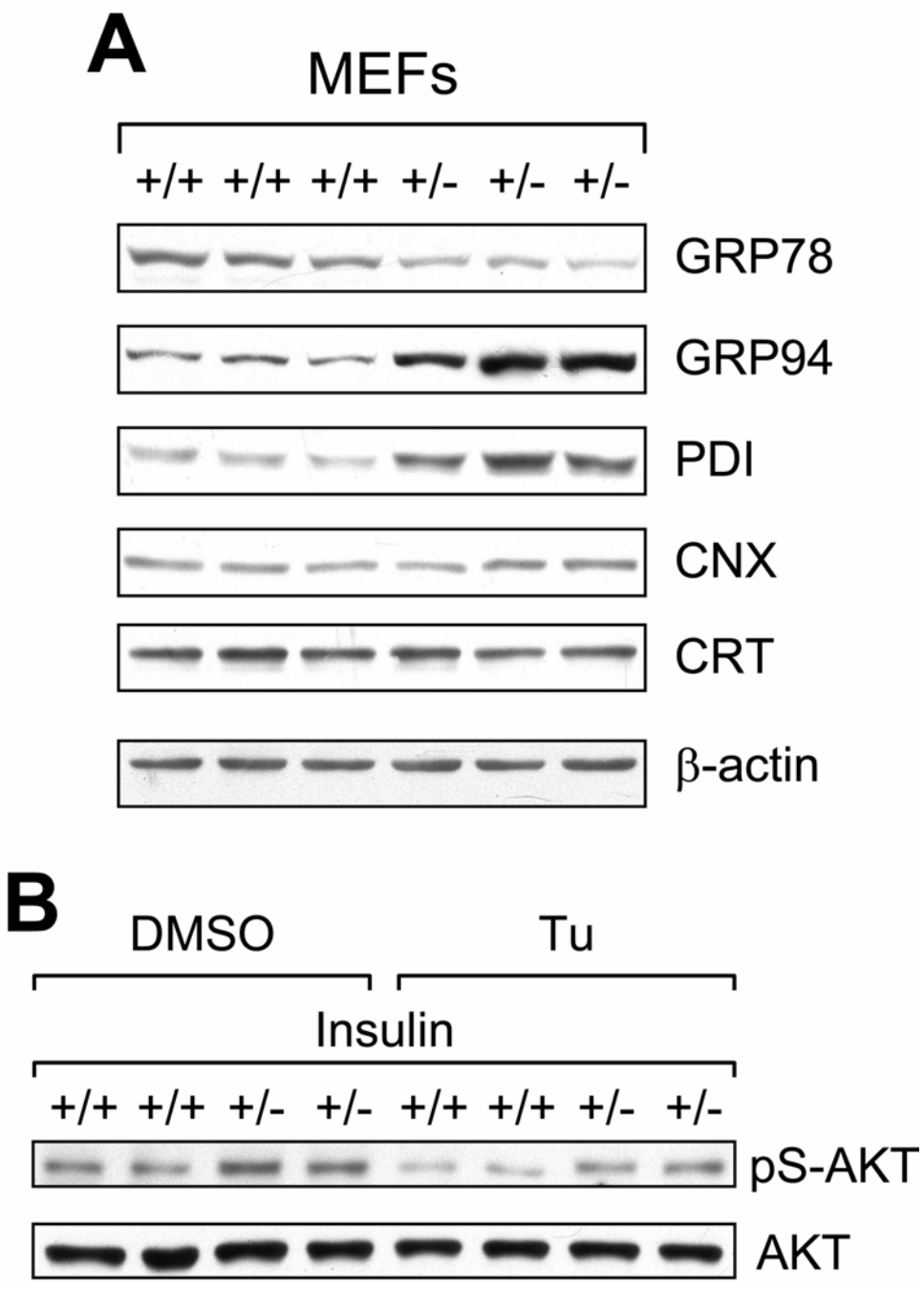
Supplementary Figure 2. Pancreatic α-cell distribution and β-cell morphology of *Grp78+/-* mice. *A*: Glucagon immunostaining on pancreas sections of mice on RD, or after 15-wk HFD. Lower panels exhibit the boxed areas within the corresponding upper panels. Numbers above scale bars indicate the represented object distance. *B*: Representative transmission electron micrograph of pancreatic β-cells in 15 wk old *Grp78+/+* and +/- mice on RD. The secretory granules (SG) containing electron dense proteins, as well as the nuclei (N), are labeled.



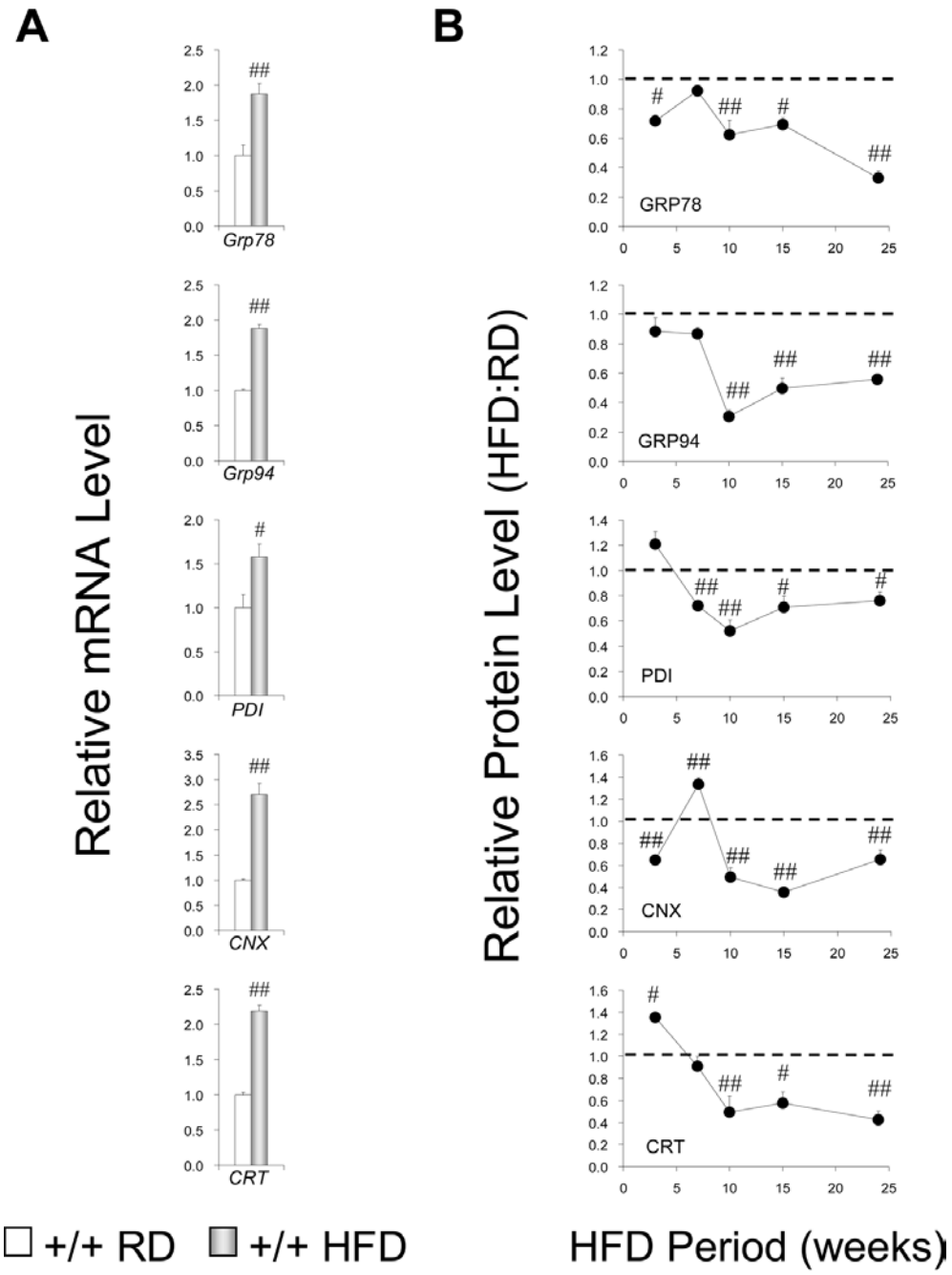
Supplementary Figure 3. Adaptive UPR signaling in WAT of *Grp78+/-* mice on HFD. Whole cell lysates were prepared from WAT of 25-week old mice on RD or after 15-week HFD (n=4-6 mice per condition). The indicated UPR signaling molecules were examined by Western blotting.



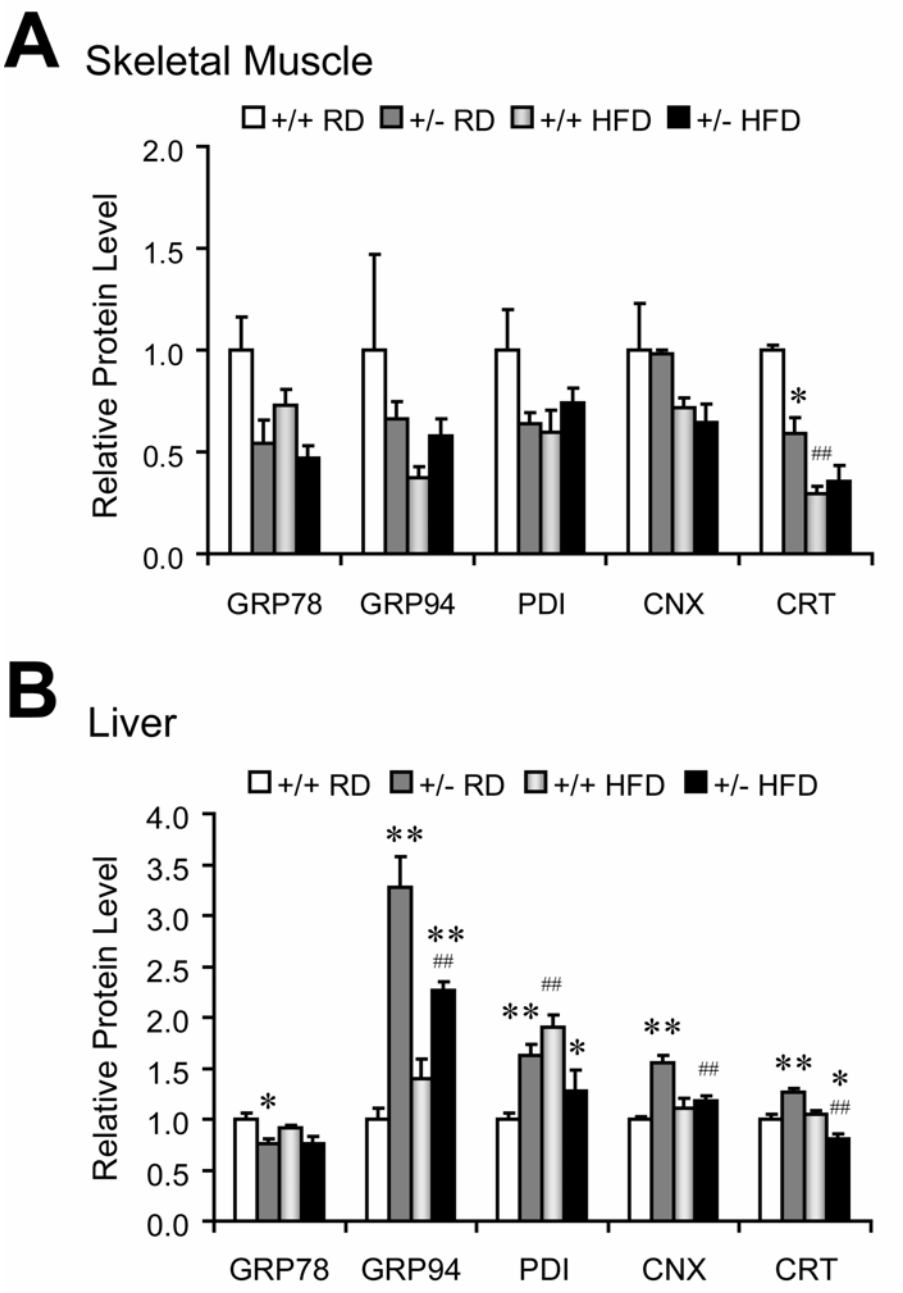
Supplementary Figure 4. Upregulation of ER chaperones GRP94 and PDI and enhancement of insulin sensitivity in immortalized *Grp78+/-* MEFs. *A*: Whole cell lysates from immortalized wild-type (+/+) and *Grp78+/-* MEFs were subjected to Western blotting for GRP78, GRP94, PDI, calnexin (CNX), and calreticulin (CRT). *B*: Immortalized *Grp78+/-* and +/+ MEFs were treated with insulin (100 nM, 15 min) following 5 h serum starvation and 3 h treatment of DMSO or tunicamycin (Tu, 10μg/mL). Whole cell lysates were subjected to Western blot for phosphorylated (Ser473) and total AKT.



Supplementary Figure 5. Posttranscriptional down-regulation of ER chaperones in WAT following HFD regimen. Analyses were performed on WAT of wild-type (*Grp78+/+*) mice (n≥3 per condition) *A*: Relative mRNA levels of the indicated ER chaperones (25-week old, 15 weeks of HFD versus RD). The mRNA levels were measured by RT-PCR and normalized against β-actin. *B*: Relative protein levels of the indicated ER chaperones were measured by Western blot (normalized against β-actin) after 3, 7, 10, 15, and 24 weeks of HFD, compared to the age-matched RD controls. The dashed lines indicate the HFD:RD ratio value of 1. Data are mean±SEM. #P<0.05, ##P<0.01 for HFD versus RD.



Supplementary Figure 6. ER chaperone levels in skeletal muscle and liver. Quantitation of ER chaperones levels in skeletal muscle (normalized against α-actin, *A*) and liver (normalized against β-actin, *B*) of *Grp78*+/+ and +/- mice on RD or after 15-week HFD. Data are presented as the mean±SEM. *P<0.05, **P<0.01 for +/- versus +/+; ## P <0.01 for HFD versus RD.



Supplementary Figure 7. *Grp78* heterozygosity protects against diet-induced obesity and insulin resistance. A model on the proposed mechanisms for the protective effects of *Grp78* heterozygosity, which may contribute to the metabolic benefits against diet-induced obesity and insulin resistance.