SUPPLEMENTARY METHODS

Materials and plasmids. 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), indomethacin (IND), insulin were purchased from Sigma. Amylose beads were from New England Biolabs. Polyclonal anti-phospho-ERK was from Cell Signalling. Anti-actin, anti-ERK, anti-HA, anti-GST were purchased from Santa Cruz. Anti-p62 was from Progen and anti-MBP from New England Biolabs. All antibodies were used according to manufacturers' instructions. The following expression plasmids were used: pGST-p62, pcDNAI-ERK1, pcDNA3-ERK2. Recombinant GST-ERK1 and GST-ERK2 were from Millipore. MBP-p62 was bacterially expressed from pMAL-c2-p62 expression plasmid. Lentiviral shRNA in the pLKO.1 vector specific for mouse ERK1, ERK2 and non-targeted control (NT) were purchased from Openbiosystems.

Immunoprecipitation experiments. For co-immunoprecipitation experiments, subconfluent 293 cells were transfected with the different plasmids by the calcium phosphate method, extracts were prepared in lysis buffer (25 mM Tris –HCl pH 8, 100 mM NaCl, 1% Triton-X100, 10% glycerol and protease inhibitors). and lysates were immunoprecipitated with monoclonal anti-HA antibody. Immunocomplexes captured with protein A-agarose beads were washed 5 times in lysis buffer followed by 3 washes with lysis buffer plus 0.5M NaCl. Endogenous immunoprecipitation of p62 was performed in the same lysis buffer.

Body composition. Whole-body composition (fat and lean mass) was measured using NMR technology (EchoMRI, Houston, TX).

Energy balance physiology measurements. Animals were allowed to acclimate to respiratory chambers for a period of 2 days. Subsequently, energy expenditure (EE) was measured for 2

consecutive days using a customized indirect calorimetry system combined with a drinking and feeding monitor and TSE ActiMot system (TSE-Systems).

Glucose-tolerance and insulin-tolerance tests. For the measurements of glucose tolerance and insulin sensitivity, mice were subjected to overnight fasting and injected intraperitoneally with 2 g glucose/kg body wt (25% D-glucose (Sigma) in 0.9% saline) for the glucose-tolerance test (GTT), and 0.75 U insulin/kg body wt (0.1 U/ml; Humalog Pen, Lily, Indianapolis, IN) for the insulin-tolerance test (ITT). Tail blood glucose levels (mg/dl) were measured by using a handheld glucometer (TheraSense Freestyle) before (0 min) and at 15, 30, 60, 90, and 120 min after injection.

Cold adaptation studies. Animals were placed in a cold-temperature environment of 4°C and body core temperature was measured using a rectal thermometer (Physitemp, Clifton, NJ). Heat production was visualized using a high-resolution infrared camera (FLIR PM280; FLIR Systems). Images of the backs of the mice were captured before exposure and 2, 4, and 6 hours after exposure to the 4°C environment. Infrared thermography images were taken from the upper half of the body to specifically visualize heat production from the BAT.

Histology. Tissues were fixed in 10% neutral buffered formalin, dehydrated in ethanol, and then transferred to xylene solution for embedding in paraffin. Sections (5 μm) were cut, mounted, and stained with H&E.

Real-time PCR. Total RNA was extracted using TRIzol reagent (Invitrogen) and RNeasy Mini Kits (Qiagen) following the manufacturers protocols. The extracted RNA was treated with DNAse (Ambion) before reverse transcription (Applied Qiagen). Reverse transcription was performed with 1 μg of total RNA. Quantitative real-time PCR was utilized to evaluate

expression levels from the cDNA by using Absolute SYBR Green real-time PCR mastermix (Thermo Fisher Scientific) and a real-time PCR thermocycler (Eppendorf). Expression levels of each gene were measured and then normalized to 18S. The primers used in the real-time PCR were as follows: PPARγ, sense 5'-GAACGTGAAGCCCATCGAGGAC-3′, antisense 5'-CTGGAGCACCTTGGCGAACA-3′; FAS, sense 5'- CTTCAACCTGGCCATGGTTTT-3', antisense 5'-GTTGGCGAAGCCGTAGTTAGTT-3'; aP2, sense 5'-AACACCGAGATTTCCTT-3', antisense 5'-ACACATTCCACCACCAG-3'.

SUPPLEMENTARY FIGURES

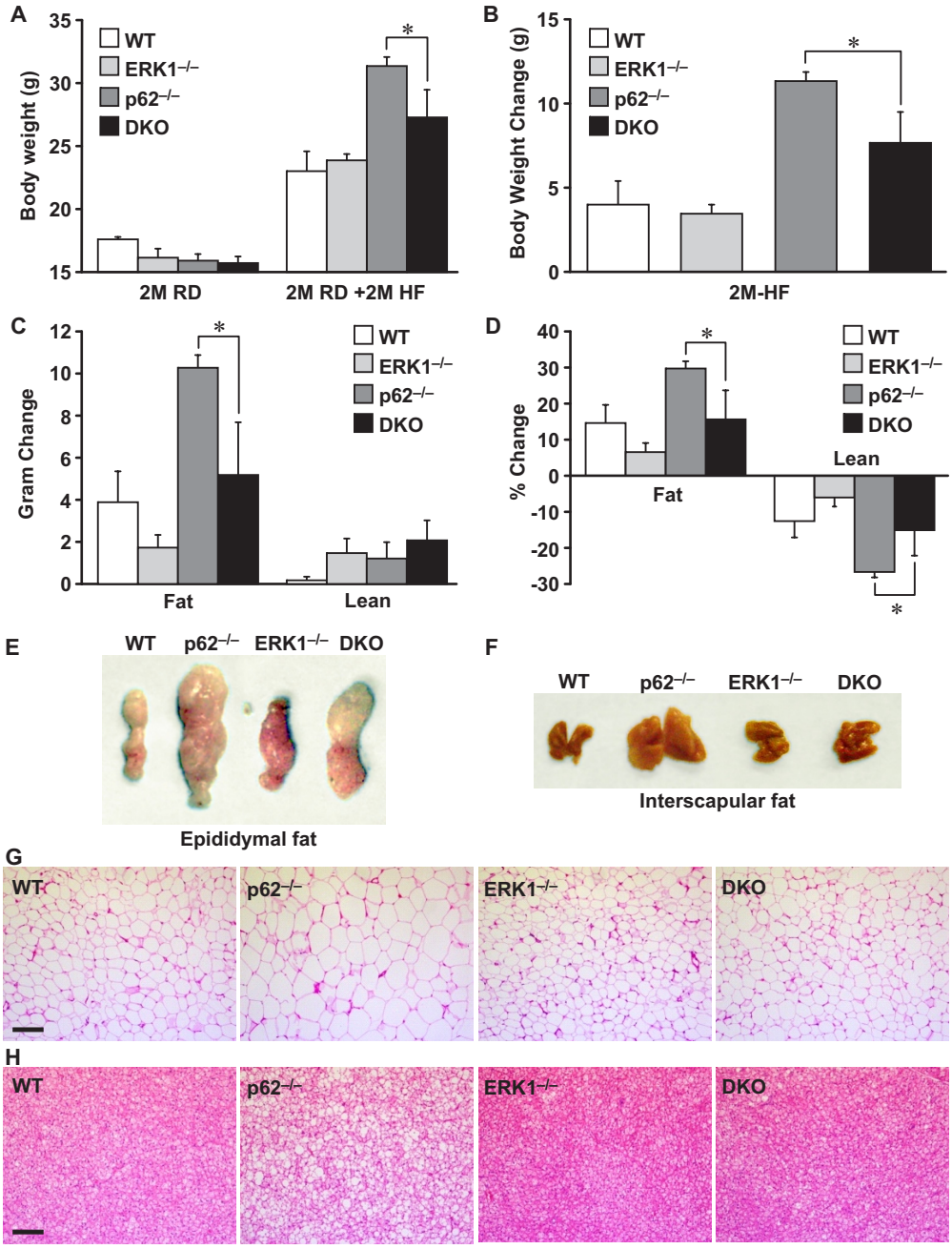

Fig S1. Decreased obesity in DKO mice fed a HFD. Wild-type (WT), p62$^{-/-}$, ERK1$^{-/-}$, and DKO mice fed a 45% high-fat diet (HFD) were tracked for 2 months, beginning at 2 months of age. (A) Body weight. (B) Changes in body weight over 2 months of HFD. (C) Grams of fat and lean mass. (D) Fat and lean mass as the percentage of total body weight. Data are mean ± S.E. of

5 to 6 animals per group. *$p < 0.05$ versus WT. (E) Epididymal adipose tissue. (F) Interscapular adipose tissue. (G) Histological analysis of epididymal adipose tissue. (H) Histological analysis of interscapular brown adipose tissue. Sections obtained from 3 different animals were stained with H&E. Scale Bar, 200 μm.

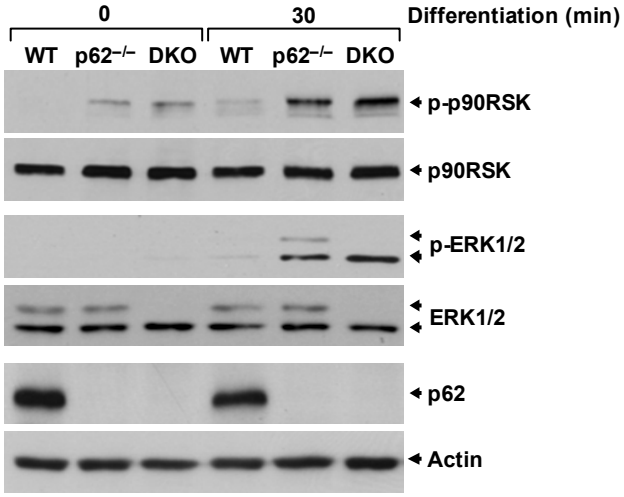

Fig S2. ERK signaling during adipogenesis in p62$^{-/-}$ MEFs. MEFs from WT, p62$^{-/-}$, and DKO mice were treated with differentiation medium to induce adipogenesis for 30 min and whole-cell extracts were prepared. Lysates were probed with the indicated antibodies.