Supplementary File

Supplementary Materials and Methods

Culture of primary fibroblast

Hap1$^{+/-}$♂ and *Hap1*$^{+/-}$♀ mice were mated to get WT and *Hap1*$^{-/-}$ newborn mice. The animals were anesthetized with pentobarbital. After 3min sterilization in 75% alcohol, mice were moved to the clean bench. The skin was cut from the newborn mice back, washed with PBS 3 times, cut into 2mm×2mm pieces, and plated on the dishes. Skin pieces were sat on the dish without media for 5min before the appropriate volume of culture media was gently added so that tissues can adhere to the culture dish. Adherent fibroblasts were observed ~2-3 days after plating and confluent wells are observed ~7-9 days after plating. At passage 1, fibroblasts were identified by anti-vimentin immunofluorescence staining (Fig. S2).

Plasma Membrane Protein Isolation

Cell membrane fraction was isolated with the Plasma Membrane Protein Isolation Kit (Bestbio, Shanghai, China) according to manufacturer's instructions. After insulin stimulation for 30min, the culture dish was quickly transferred to ice. The culture medium was aspirated, and cells were washed twice with cold PBS. 200μl of solution A was added into dish and cells were collected into a 1.5mL tube with the cell scraper. The cell lysate was repeatedly blown up for 30-50 times with the 1mL syringe to completely lyse the cells. The lysate was centrifuged at 1000g for 5min at 4°C. The supernatant was a mixture of cytoplasmic protein and membrane protein, and the precipitate was nuclear protein. The supernatant was inhaled into a new tube, and 4μl of solution C was added in and mixed by repeated blowing. The mixture was shaken at 4°C for 30min to fully combine the solution C with the membrane protein, and then heated in a 37°C water bath for 10min to precipitate the solution C. after centrifugation at 1000g for 3min at 37°C, the solution was divided into two layers. The upper liquid was carefully injected into a new tube to obtain cytoplasmic protein, and the lower layer was mixed with 50μl of solution D to fully dissolve the lower fraction to obtain membrane protein for subsequent western blot experiments.

Supplementary Figures

Figure S1. Gene identification results. The wild-type band was about 550bp, and the knockout band was about 350bp.The genotype of samples with bands only at 550bp was WT, the genotype of samples with bands at 550bp and 350bp was *Hap1*$^{+/-}$, and the genotype of samples with bands only at 350bp was *Hap1*$^{-/-}$. Blank is a blank control without DNA sample.

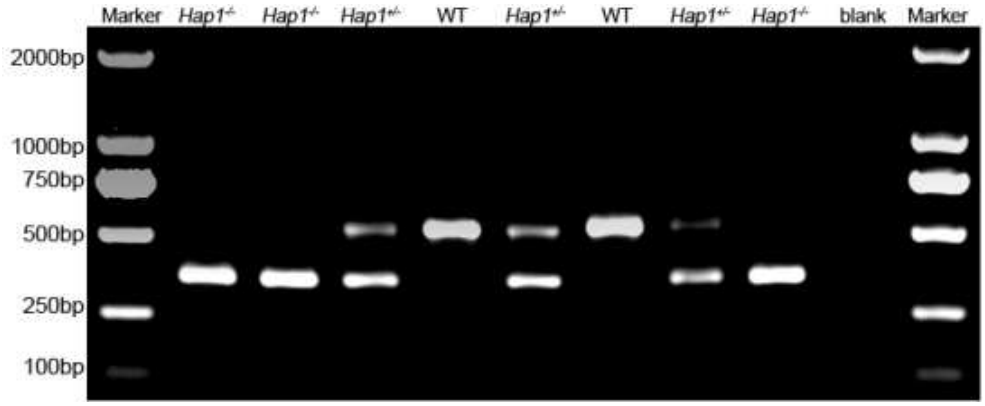

Figure S2. The primary fibroblasts and fibroblast-derived adipocytes. (**A**) P0 mouse back skin was cut for explant culture, and the bright field photo on the 6th day of culture was shown. Scale bar, 50μm. (**B**) Immunofluorescence staining of rabbit anti-Vimentin antibody in primary fibroblasts. Vimentin (red) was mainly located in the cytoplasm and the signal distribution was filamentous. The nuclei (blue) were stained with DAPI. Scale bar, 50μm. (**C**) Bright filed photos of adipogenic process induced from primary fibroblasts. After induction for 1 day (d1), cells became elongated and refractive index increased. After induction for 3 days (d3), cells retracted. Significant lipid droplet was observed 5 days after induction (d5). The black arrow showed the differentiated fibroblasts with lipid droplets. Scale bar, 50μm. (**D**) Adipocytes induced from primary fibroblasts were stained with oil red at d6. The red refractive signal was lipid droplets. The black arrow showed the location of the nucleus. Scale bar, 50μm.

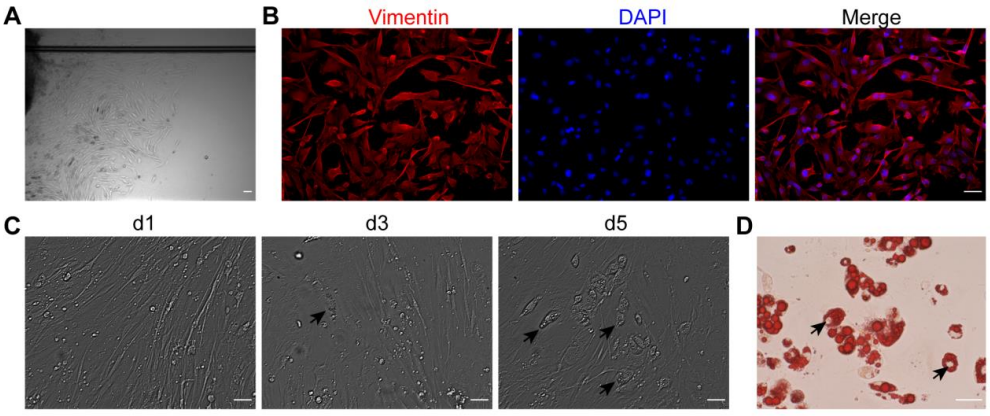

Figure S3. The positive and negative control of HAP1 antibody. (**A**) Western blotting results of brain tissues from different genotype newborn mice detected by mouse anti-HAP1 antibody (NB110-74569, 1:1000, Novus). (**B**) Western blotting results of brain tissues from different genotype newborn mice detected by goat anti-HAP1 antibody (sc-12556, 1:1000, Santa Cruz). (**C**) The immunohistochemistry results of mouse anti-HAP1 antibody (left) and mouse IgG control (right) of WT mouse hypothalamus. Scale bar, 50μm. (**D**) The immunohistochemistry results of mouse anti-HAP1 antibody of WT adipocytes and *Hap1*$^{-/-}$ adipocytes.

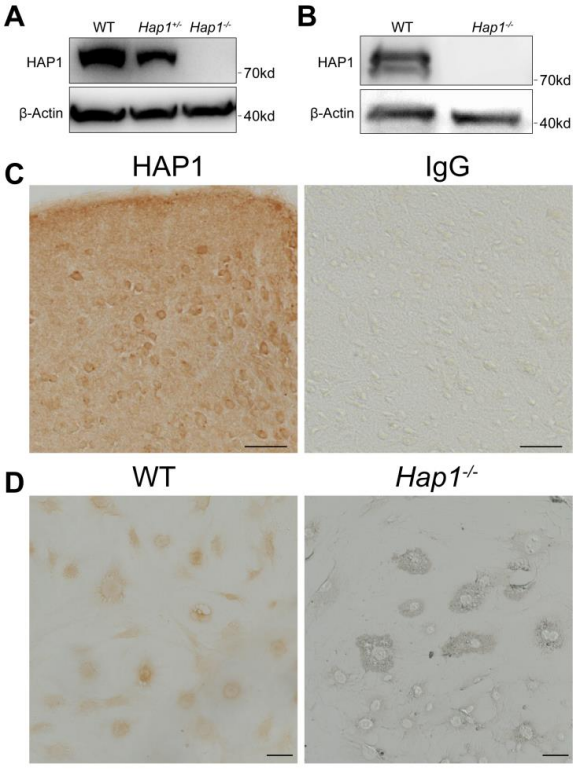

Figure S4. Diet-induced diabetes in mice. WT mice were fed with HFD and glucometabolic parameters were monitored. (**A**) Kinetics of body weight increase rate of HFD fed mice (n=6) and normal diet (ND) fed mice (n=4). $p<0.001$ for diet effect across all time points (two-way ANOVA). (**B**) Blood glucose levels of mice fasting for 16h at 40 weeks (n=9 for HFD fed mice and n=4 for ND fed mice), $p<0.0001$. (**C**) Relative blood glucose levels and AUC during a GTT of mice at 25 weeks (n=10 for HFD fed mice and n=4 for ND fed mice), $p=0.0003$ for diet effect, $p=0.0026$ for diet and time interaction (two-way ANOVA). (**D**) Relative blood glucose levels and AUC during an ITT of mice at 25 weeks (n=7 for HFD fed mice and n=4 for ND fed mice), $p=0.0006$ for diet and time interaction (two-way ANOVA).

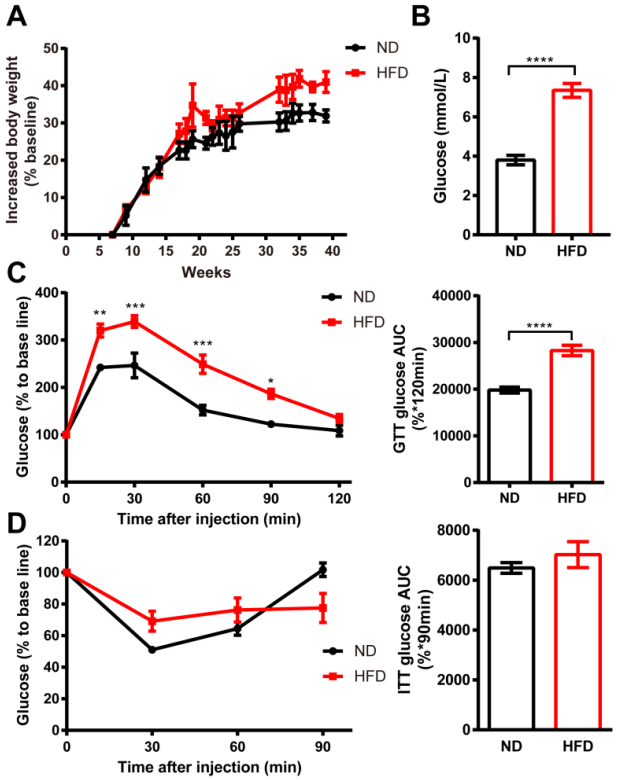

Figure S5. Regulation of GLUT4 translocation in adipose tissue by HAP1. Immunofluorescence staining of GLUT4 (left) with cultured primary adipocytes from WT and *Hap1*$^{-/-}$ mice after 30min insulin stimulation following 2h starvation and line-scan analysis corresponding to each group (right). Scale bar, 50μm.