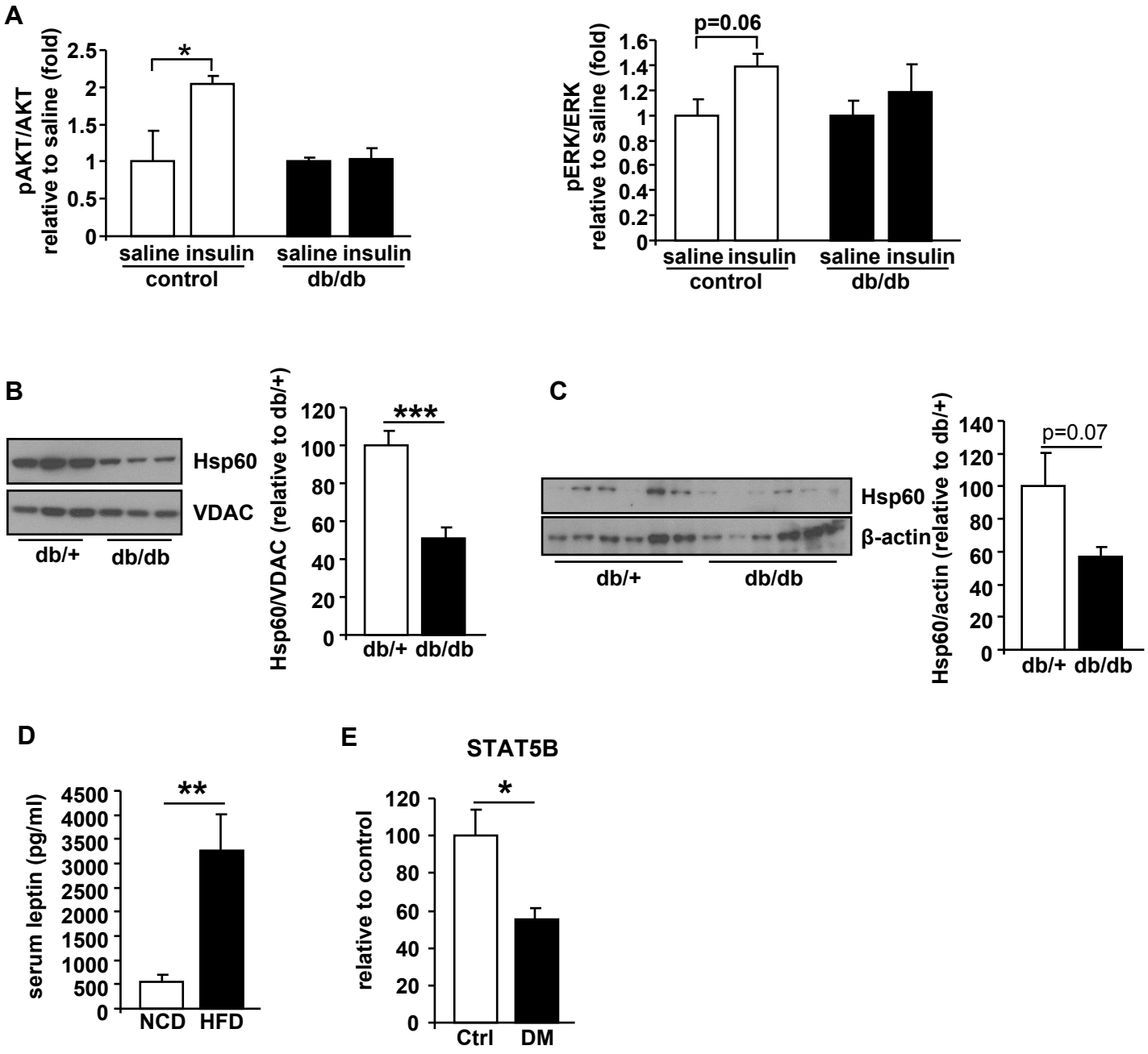
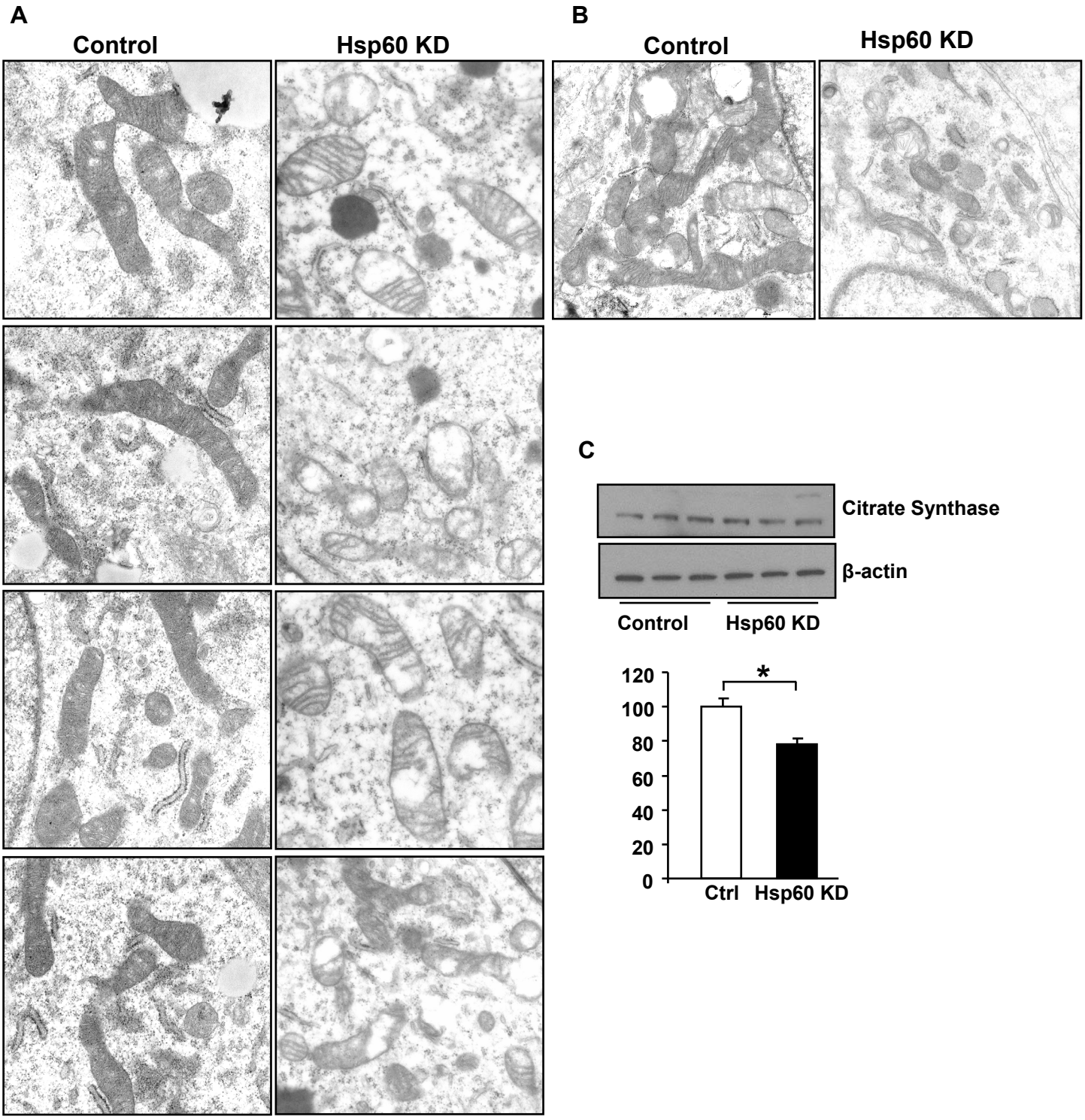


Suppl Figure 1

Hsp60 reduction is associated with central insulin resistance

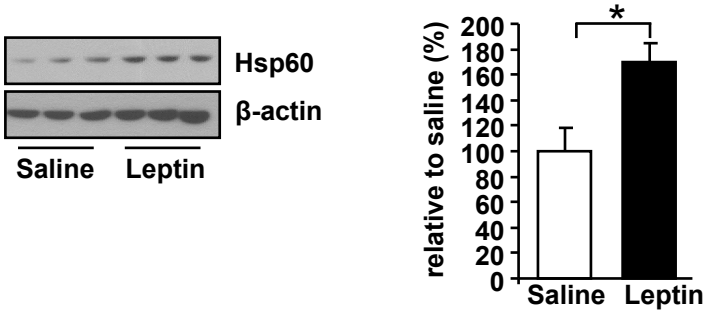
(**A**) Densitometric analysis of AKT and ERK activation after insulin stimulation in the hypothalamus of control and db/db mice (n=3-5). (**B**) Western blot and densitometric analysis of Hsp60 of isolated mitochondria from hypothalami of db/+ and db/db mice (each n=6). VDAC served as loading control for mitochondrial content. (**C**) Western blot and densitometric analysis of cytoplasmic Hsp60 of hypothalami from db/+ and db/db mice (each n=6). β-actin served as loading control. (**D**) Serum leptin levels of mice fed a NCD or HFD for 14 weeks (n=4-5). (**E**) Gene expression analysis of STAT5B of hypothalami from control and type 2 diabetes mellitus patients (n=3-4). Displayed values are means ± S.E.M.; *, $p \leq 0.05$; ***, $p \leq 0.001$



Suppl Figure 2

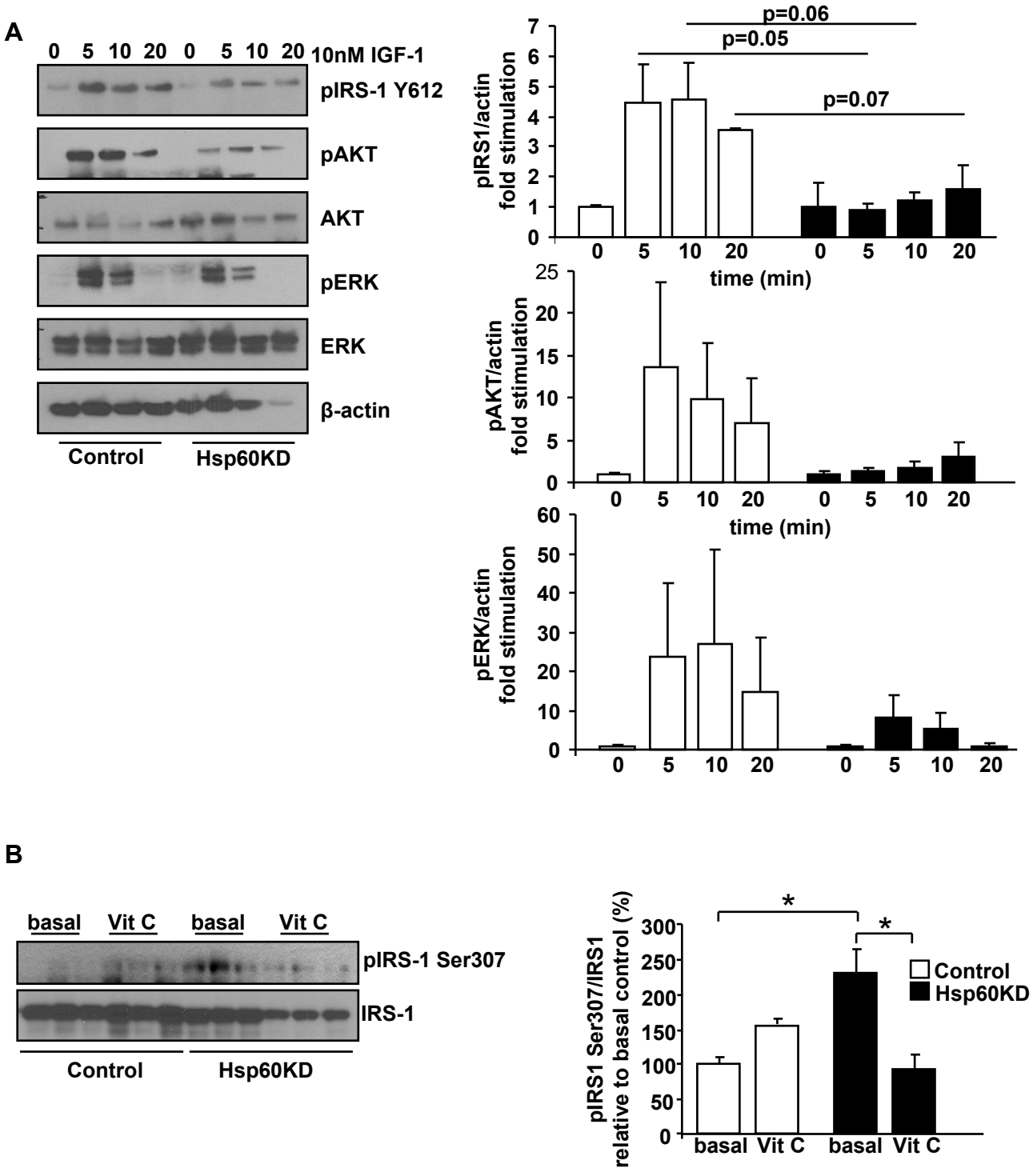
Hsp60 knockdown causes mitochondrial dysfunction, Electron microscopic pictures of mitochondria from control and Hsp60 KD in two different neuronal cell lines (**A**) N25/2 (Total magnification 1:46550) and (**B**) GT1-7 (Total magnification 1:34500) (**C**) Western blot and densitometric analysis of citrate synthase in control and Hsp60 KD cells (each n=3). β-actin served as a loading control. Displayed values are means ± S.E.M.; *, $p \leq 0.05$.

A



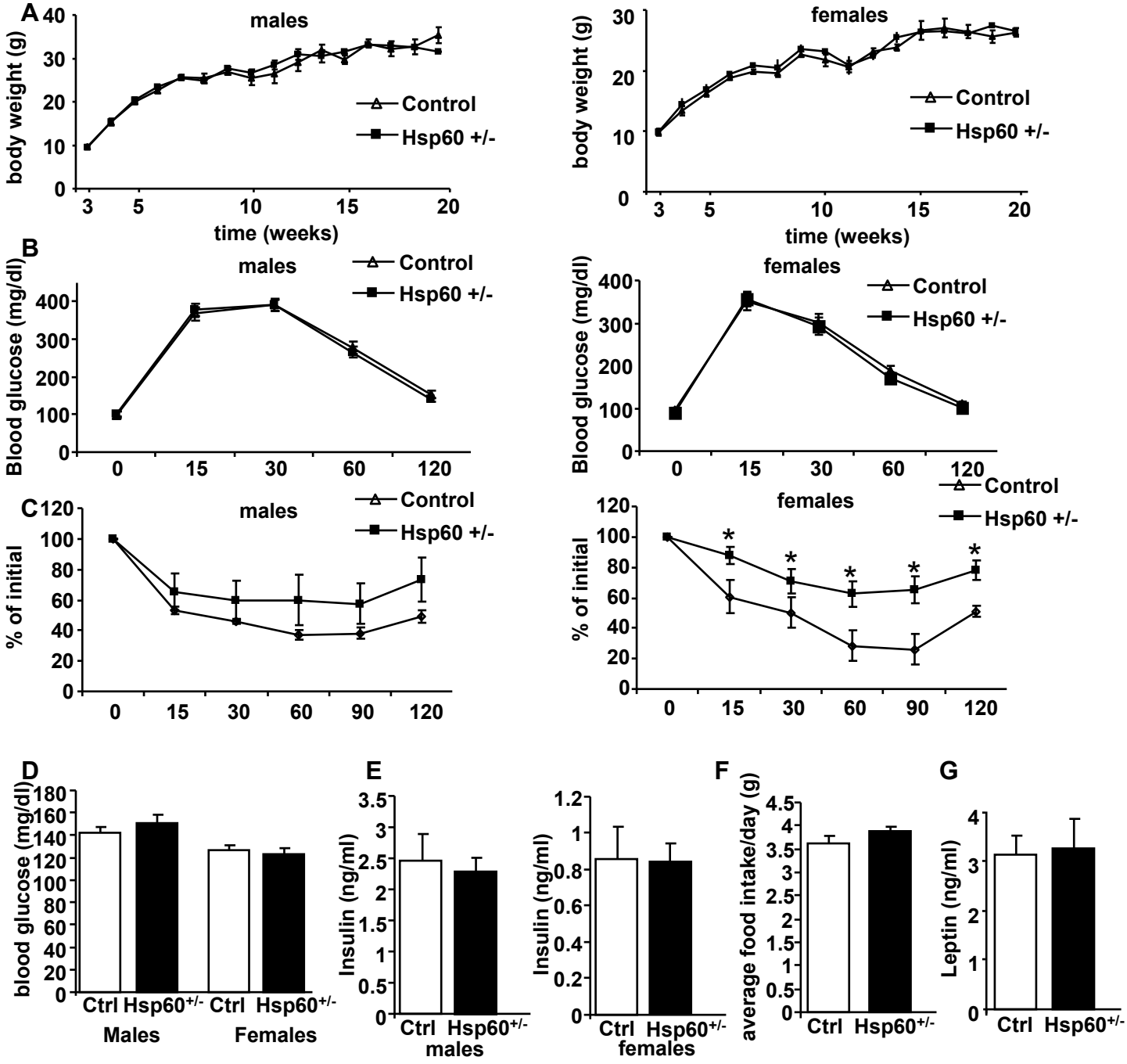
Suppl Figure 3

Hypothalamic Hsp60 expression in C57Bl/6 mice (**A**) Western blot and densitometric analysis of Hsp60 of hypothalami from mice treated with saline or leptin for 2h (each n=3-6). β-actin served as a loading control. Displayed values are means ± S.E.M.; *, $p \leq 0.05$.



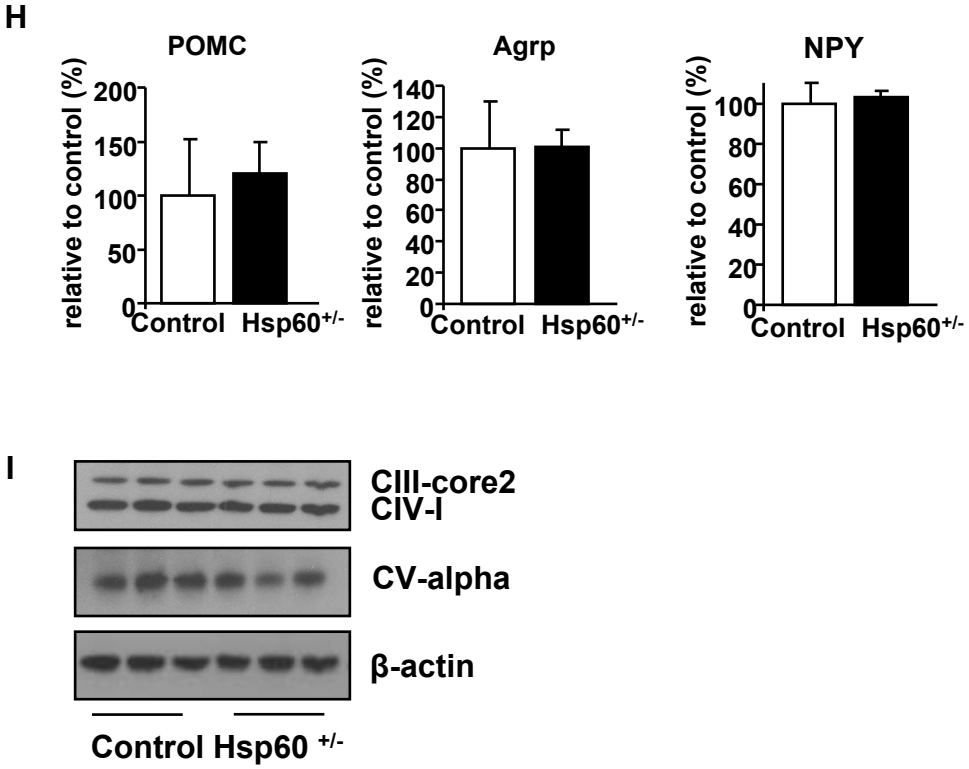
Suppl Figure 4

Insulin and IGF-1 resistance in Hsp60 KD cells. (**A**) Western blot and densitometric analysis of 10nM IGF-1 stimulated phosphorylation of IRS-1, AKT and ERK in control and Hsp60 KD cells (n=5). (**B**) Western blot and densitometric analysis of phosphorylated IRS1 Ser307 of control and Hsp60 KD cells treated with vitamin C. Displayed values are means ± S.E.M.; *, $p \leq 0.05$.



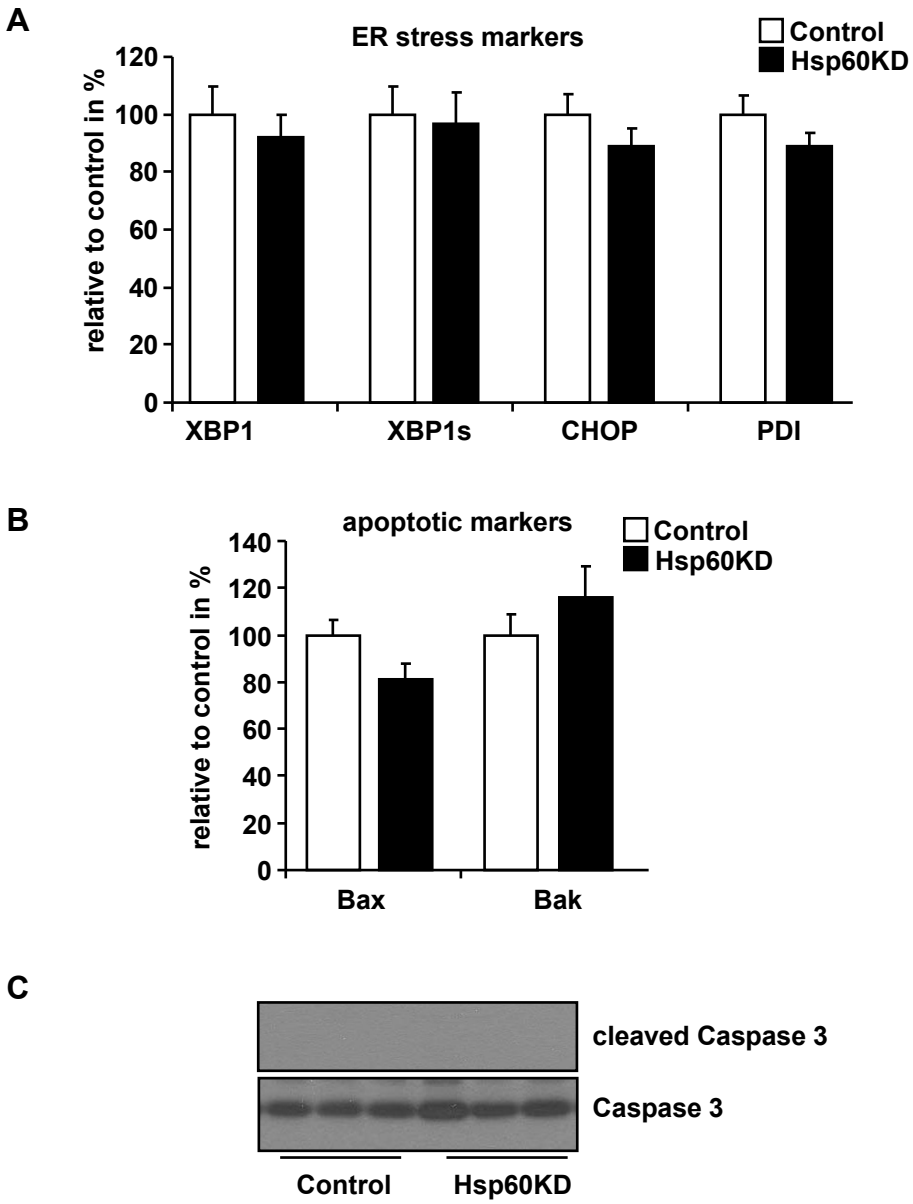
Suppl Figure 5

Metabolic phenotype of Hsp60 heterozygous mice. (**A**) Body weight curve of control and Hsp60$^{+/-}$ male and female mice over the time of 20 weeks (n=11-13). (**B**) Glucose tolerance test of 12-week old control and Hsp60$^{+/-}$ male and female mice (n=10-13). (**C**) Insulin tolerance test of 16-week old control and Hsp60$^{+/-}$ male and female mice (n=5-9). (**D**) Random blood glucose levels of 20-week old control and Hsp60+/- male and female mice (n=5-8). (**E**) Serum insulin levels of 16-week old control and Hsp60+/- male and female mice (n=13-16). (**F**) Average food intake of 20-week old control and Hsp60$^{+/-}$ male mice (n=5-11). (**G**) Serum leptin levels of 16-week old control and Hsp60+/- male mice (n=10).



Suppl Figure 5

Metabolic phenotype of Hsp60 heterozygous mice. (**H**) Hypothalamic expression of anorexigenic and orexigenic neuropeptides in control and Hsp60$^{+/-}$ mice (n=6-8). (**I**) Western blot analysis of mitochondrial proteins CV alpha, CIV-I and CIII-core 2 of dissected hypothalami of control and Hsp60$^{+/-}$ mice (each n=3). β-actin served as a loading control. (**J**) Densitometry of western blot analysis. Values are means ± S.E.M.; *, $p \leq 0.05$.



Suppl Figure 6

Acute downregulation of Hsp60 in the hypothalamus does not induce ER stress or apoptosis. (**A**) Gene expression analysis of ER stress and (**B**) pro-apoptotic markers in hypothalamic samples of control and Hsp60 KD mice (n=7-8). (**C**) Western blot analysis of cleaved and total caspase 3 in hypothalamic samples control and Hsp60 KD mice (n=7-8).