Supplementary Fig 1: (A) mRNA expression of *Acadl* (long-chain acyl-CoA dehydrogenase), *Acadm* (medium-chain acyl-CoA dehydrogenase), and *Hadha* (long-chain hydroxyacyl-CoA dehydrogenase) in livers of fed and fasted control and *hadh*<sup>-/-</sup> mice. (B) Western blots analysis of lysates of *hadh*<sup>+/+</sup> and *hadh*<sup>-/-</sup> livers were performed with an anti-HADHA antibody. An antibody against GAPDH was used as loading control. (C) Western blot analysis of HADHA in lysates of heart, skeletal muscle, brown adipose tissue and isolated islets of *hadh*<sup>+/+</sup> and *hadh*<sup>-/-</sup> mice. Quantification of HADHA protein expression as detected by Western blotting is shown in the right panels of B and C.

9 Supplementary Fig. 2: No alterations of body weight and body fat content of hadh<sup>-/-</sup> mice that were
10 kept on a standard diet. Body weight development of male hadh<sup>+/+</sup> and hadh<sup>-/-</sup> mice fed a standard
11 diet (SD; upper panel). Body fat mass of male hadh<sup>+/+</sup> and hadh<sup>-/-</sup> mice fed a SD (lower panel). Data
12 represent mean of 17- 20 mice ± SE.

Supplementary Fig. 3: No changes in CD36 expression in BAT of hadh<sup>+/+</sup> and hadh<sup>-/-</sup> mice kept at 22°C or 4°C. Western blot analyses of lysates from BAT of hadh<sup>+/+</sup> and hadh<sup>-/-</sup> mice (HFD) at the age of 11 weeks were performed with an antibody against the fatty acid transporter CD36. An antibody against GAPDH was used as loading control.

**Supplementary Fig. 4:** Detection of ketone bodies in plasma of  $hadh^{+/+}$  and  $hadh^{-/-}$  mice that were 20 kept on HFD. Plasma samples of mice that were randomly fed, fasted over night or kept for 3h at 4°C 21 were used for measuring ketone bodies as described in Material and Methods. (n= 7.11, #, p<0.001)





Liver



# Isolated islets of Langerhans

hadh+/+

hadh-/-

– – – – HADHA **β-actin** 







### **CD36**

### GAPDH

Ketone bodies [umol/l]	1600						
	1400						
	1200						
	1000						
	800						
	600						
	400						
	200			<u> </u>			
	0						
		fed					



## fasted



## 3h 4°C