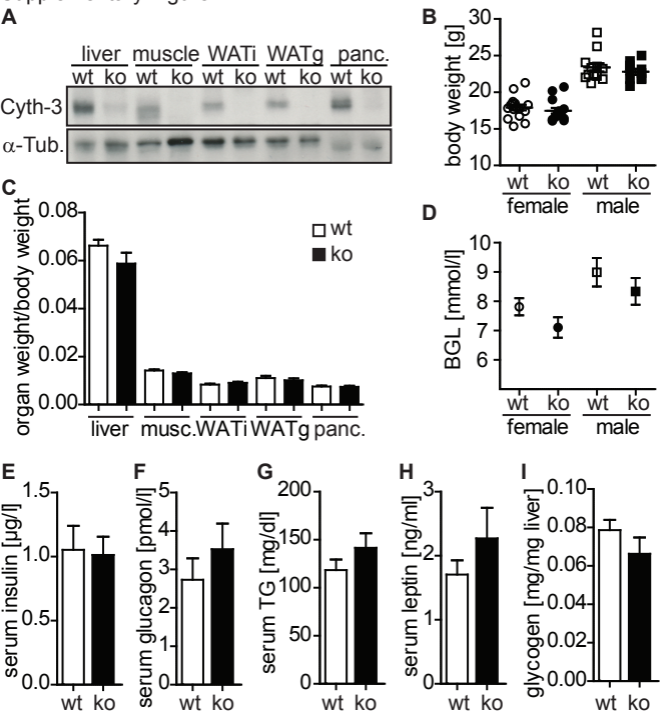


**Cytohesin-3 is required for full insulin receptor signaling and
controls body weight via lipid excretion**

Bettina Jux, Dominic Gosejacob, Felix Tolksdorf, Christa Mandel, Michael Rieck,

Angrit Namislo, Alexander Pfeifer, Waldemar Kolanus

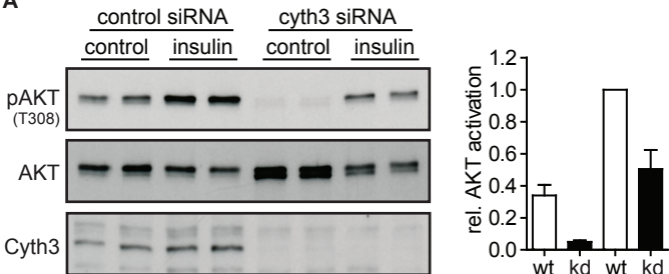
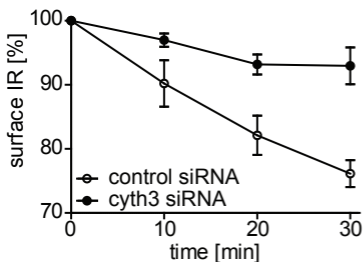
Supplementary Figure 1



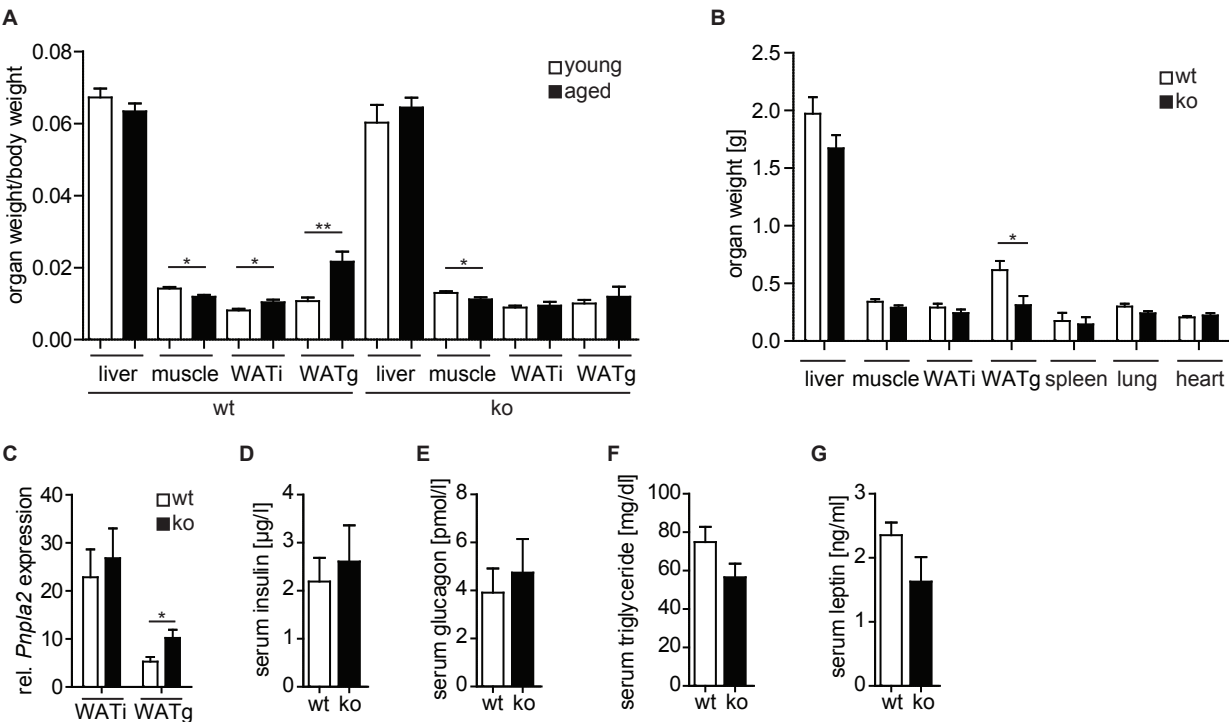
Suppl. Figure 1: Young adult $cyth3^{-/-}$ mice have a normal metabolic phenotype.

Male and female mice at the age of 7-12 weeks were phenotypically analyzed. A) A representative western blot analysis of the cytohesin-3 protein expression in liver, skeletal muscle, inguinal subcutaneous white adipose tissue (WATi), intra-abdominal gonadal white adipose tissue (WATg) and pancreas (panc.) is shown. Body weight at the age of 8-9 weeks ($n \geq 10$) (B), organ weight normalized to body weight from male mice ($n = 7-9$) (C), blood glucose levels (BGL) in non-fasted mice at the age of 8-9 weeks ($n \geq 12$) (D), serum levels of insulin ($n = 8$) (E), glucagon ($n = 7$) (F), triglycerides (TG; $n = 5$) (G), leptin ($n = 6$) (H) and liver glycogen ($n = 6$) (I) are given from male and female wt (white symbols and bars) and $cyth3^{-/-}$ (ko, black symbols and bars) mice as bar graphs of means + SEM or as individual data points \pm SEM.

Supplementary Figure 2

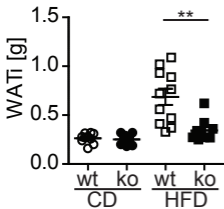
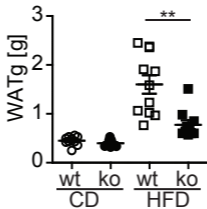
A**B**

Supplementary Figure 2: IR signaling and internalization in HepG2 cells is impaired after cyth3-knockdown. **A**) Control siRNA (wt) or Cyth3 siRNA (kd) treated HepG2 cells were stimulated with 100nM insulin. Ten minutes later cells were harvested and activation of AKT was determined as a ratio of phosphorylated to total protein levels. Representative Western blot analyses are shown. The activation was normalized to insulin-stimulated wt cells (n = 4). **B**) IR internalization after indicated time points of insulin stimulation was measured by FACS and calculated in comparison to IR surface expression before stimulation (= 100%). Results are given in means \pm SEM (n = 5).



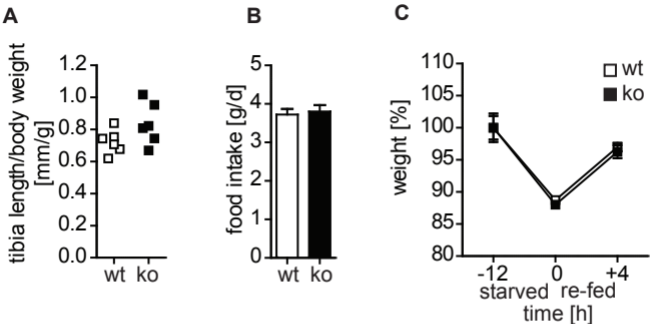
Supplementary Figure 3: Phenotypic analysis of two years old *cyth3^{+/+}* (wt) and *cyth3^{-/-}* (ko) mice. Comparison of organ weights relative to body weight from young (white bars) and aged mice (black bars) of wt and ko mice ($n \geq 6$) (A). Total organ weights of aged wt (white bars) and ko mice (black bars) ($n = 6$) (B). Expression of *Pnpla2* in adipose tissues was analyzed by PCR. The expression was normalized to *Hprt* ($n = 5-6$) (C). Serum levels of insulin ($n = 6$) (D), glucagon ($n = 5$) (E), triglycerides ($n = 6$) (F), and leptin ($n = 6$) (G) are given from aged wt (white bars) and ko (black bars) mice. Results are given as bar graphs of means + SEM (*, $p < 0.05$; **, $p < 0.01$).

Supplementary Figure 4

A**B**

Supplementary Figure 4: Fat pad weight is reduced in HFD-fed *cyth3*^{-/-} mice. Inguinal subcutaneous (WATi; **A**) and intra-abdominal gonadal white adipose tissue (WATg; **B**) from wt (white symbols) and ko mice (black symbols) was weighed after 6 weeks of HFD (squares) and CD (circles), respectively. Results are given as individual data points \pm SEM (**, $p < 0.01$).

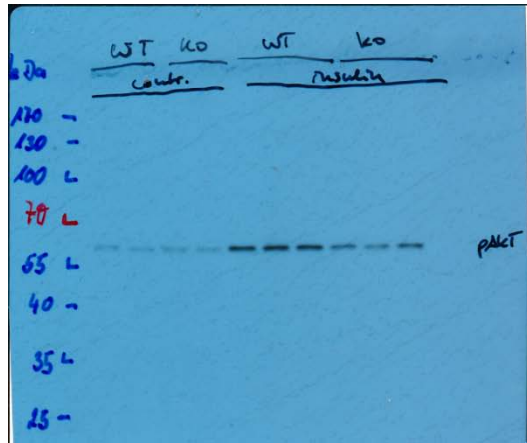
Supplementary Figure 5



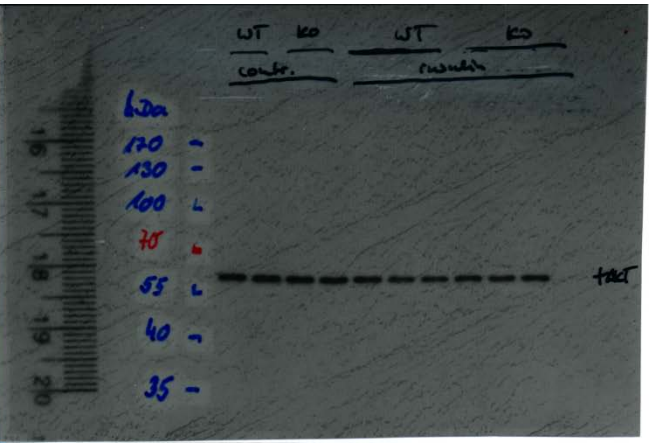
Supplementary Figure 5: Size and food uptake are similar between $cyth3^{+/+}$ (wt) and $cyth3^{-/-}$ (ko) mice. **A**) Mouse-size was determined by tibia length in relation to body weight of 8-9 weeks old mice ($n = 6$). **B**) Daily food intake of wt and $cyth3^{-/-}$ mice ($n = 6$). **C**) Weight loss and gain of wt (white squares) and $cyth3^{-/-}$ (ko, black squares) mice after a starvation period of 12 hours (-12) and subsequent re-feeding for four hours (+4) ($n \geq 12$) Results are given in means \pm SEM.

Full western blot images for Figure 1

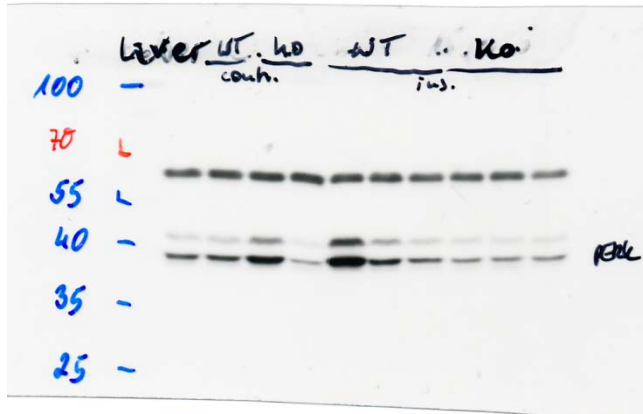
liver pAKT^{T308}
 wt ko wt ko
 control insulin



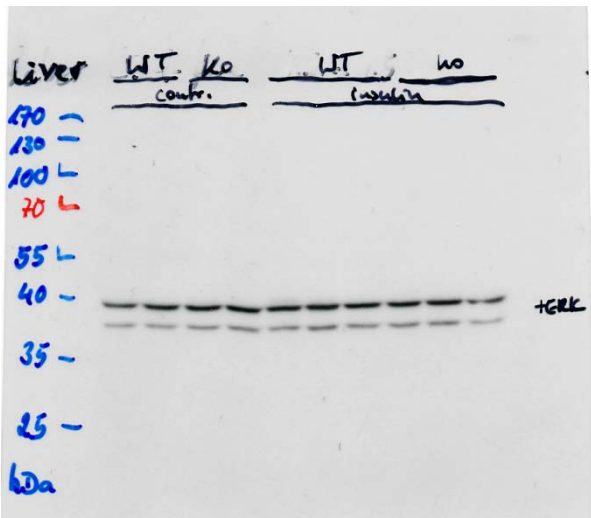
liver tAKT
 wt ko wt ko
 control insulin



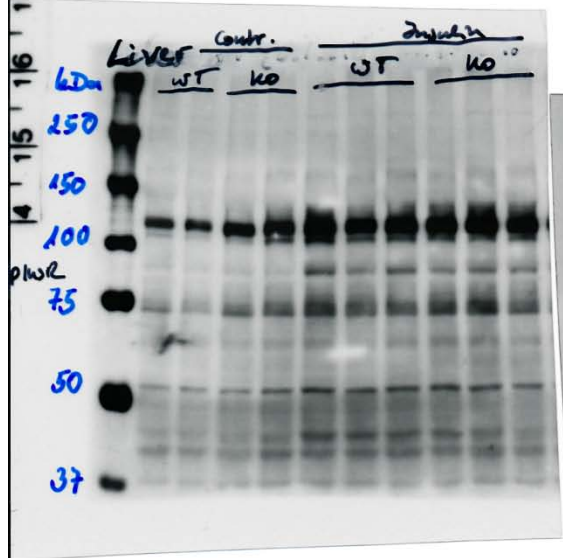
liver pERK^{T202/204}
 wt ko wt ko
 control insulin



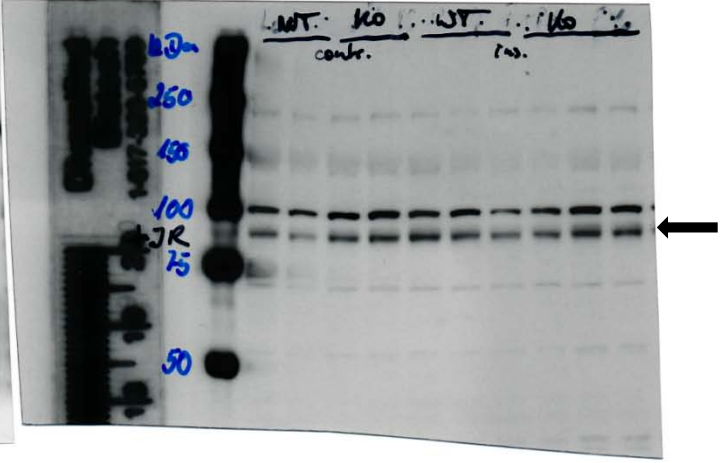
liver tERK
 wt ko wt ko
 control insulin

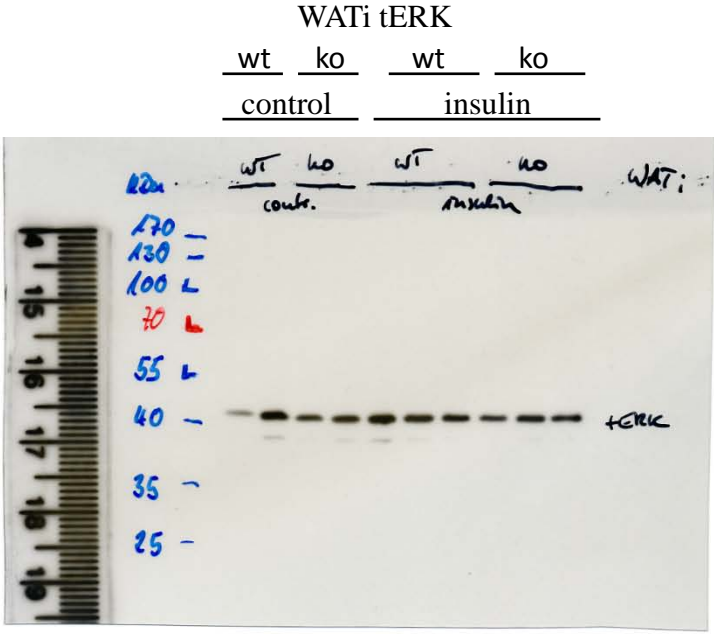
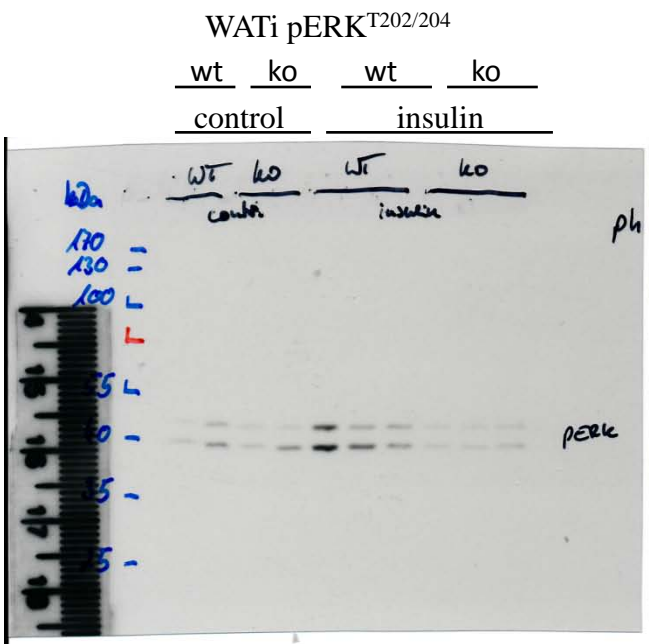
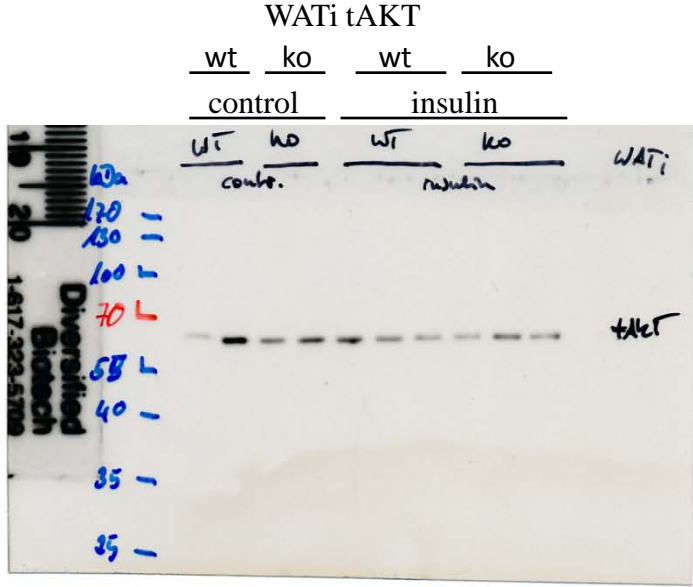
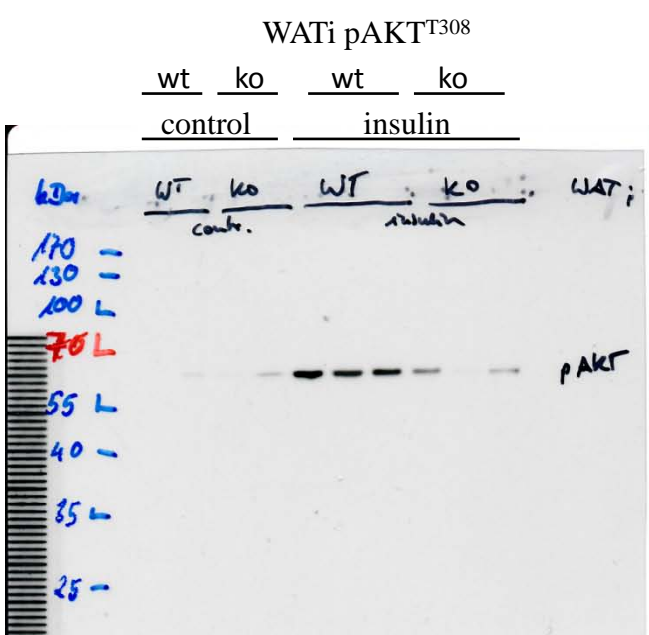


liver pIR^{Y1162/3}
 wt ko wt ko
 control insulin



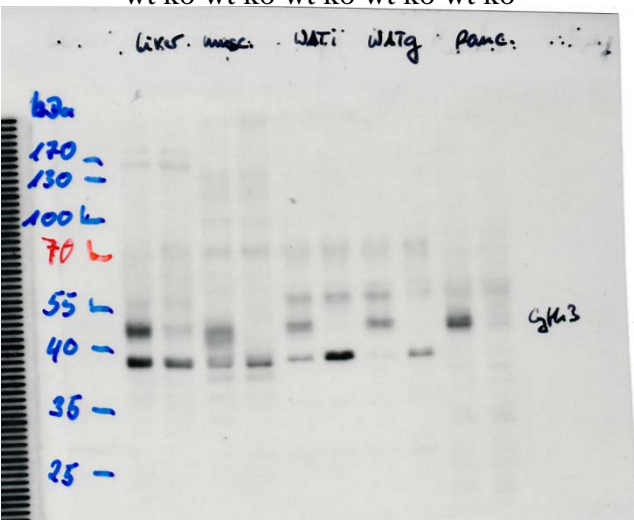
liver tIR
 wt ko wt ko
 control insulin





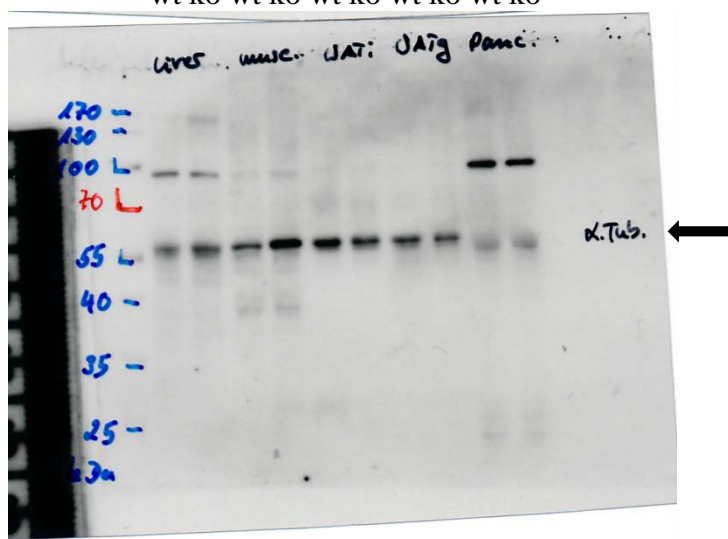
Cytohesin-3

liver musc. WATi WATg panc.
wt ko wt ko wt ko wt ko wt ko



α -Tubulin

liver musc. WATi WATg panc.
wt ko wt ko wt ko wt ko wt ko



Full western blot images for Supplementary Figure 2

control siRNA Cyth3 siRNA
contr. insulin contr. insulin

