SUPPLEMENTARY MATERIALS

Nilsen et al. 3-Hydroxyisobutyrate, a strong marker of insulin resistance in type 2 diabetes and obesity that modulates white and brown adipocyte metabolism

Supplementary Tables

Supplementary Table 1. Nucleotide sequences used for silencing RNA (siRNA*) transfection experiments.

Name	Target Sequence (5 → 3)	Manufacturer
siRNA J-040266-09	UCUAAAAGAUGUUACGGAU	Dharmacon
siRNA J-040266-10	AGGCUGUGGAGGCGUCAUA	Dharmacon
siRNA J-040266-11	CUGAAUAUGAUCCGGCAGA	Dharmacon
siRNA J-040266-12	CAUCAUAAAGGGAGCCGGA	Dharmacon

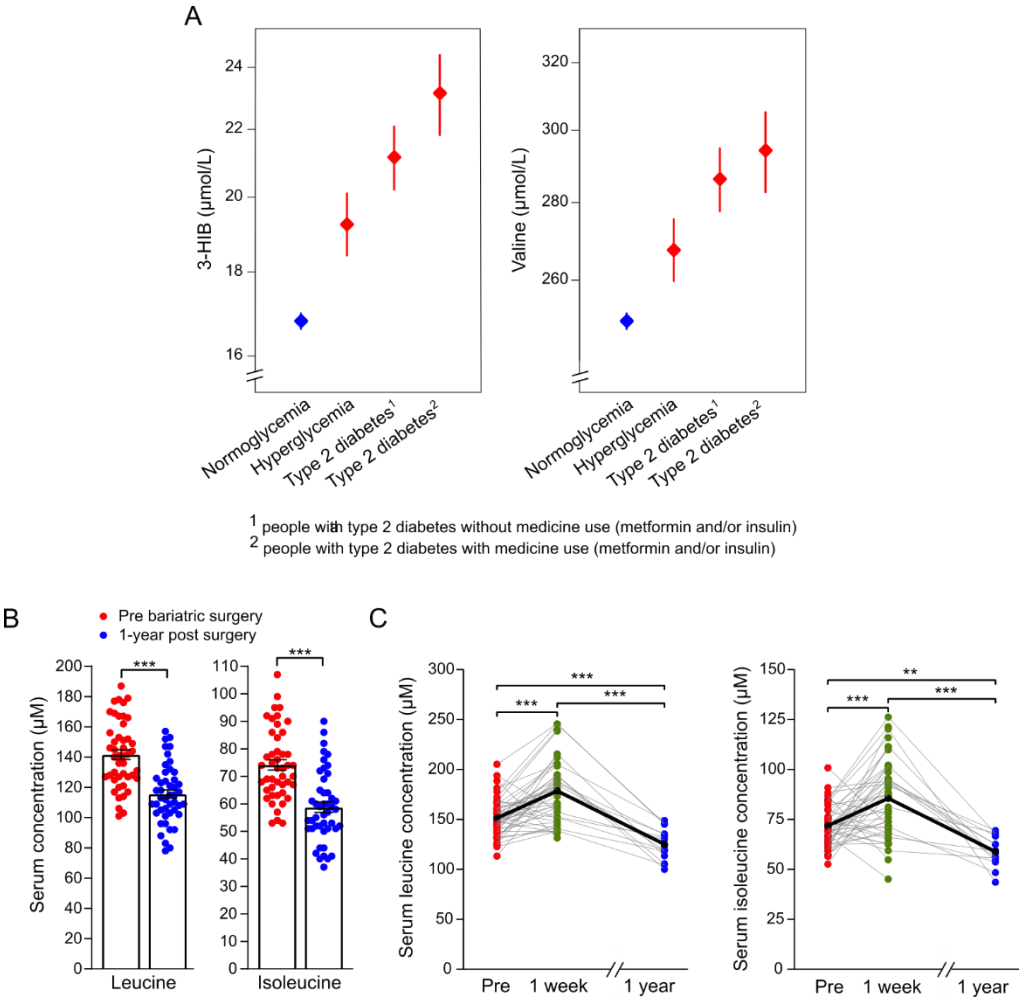
* SMART Pool ON-TARGETplus Mouse Hibch siRNA (L-040266-01-0010, Dharmacon)

Supplementary Table 2. Primers for quantitative real-time PCR.

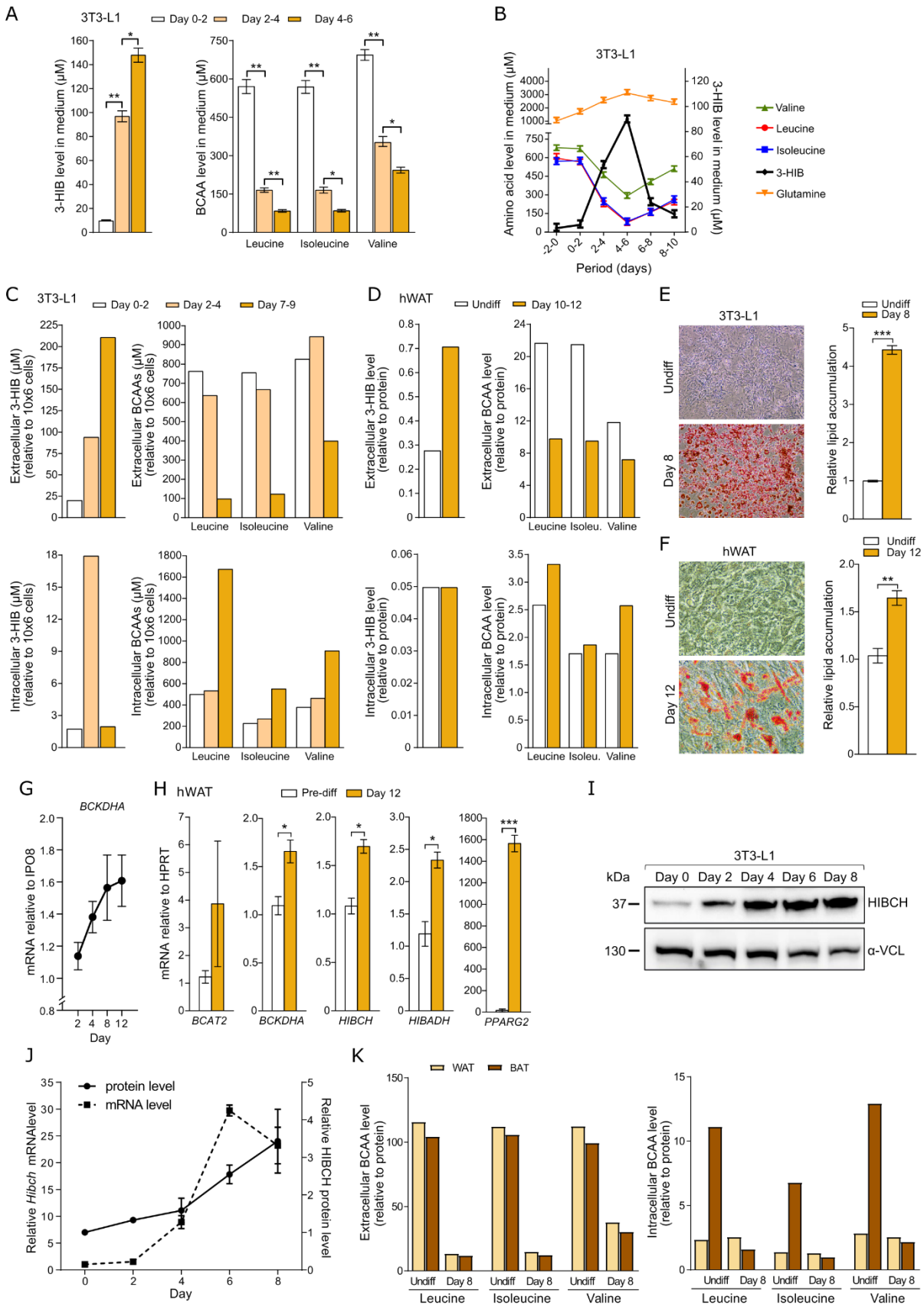
Primer	Gene name	Sequence (5 → 3)
Bcat2	branched chain amino transferase 2, mitochondrial	F: CTCATCCTGCGCTTCCAG R: TCACACCCGAAACATCCAATC
Bckdha	branched chain keto acid dehydrogenase E1, alpha polypeptide	F: ATCTACCGTGTCATGGACCG R: ATGGTGTTGAGCAGCGTCAT
Bckdhb	branched chain keto acid dehydrogenase E1 subunit beta	F: AGCTATTGCGGAAATCCAGTTT R: ACAGTTGAAAAGATCACCTGAGC
Glut4	glucose transporter type 4	F: TGTCGCTGGTTTCTCCAACT R: GCTCATGGCTGGAACTCG
Hibadh	3-hydroxyisobutyrate dehydrogenase	F: GCAGCGGTGTGTTCTAGGTC R: ACACGTCATAGAGGATGAGTGG
Hibch	3-hydroxyisobutyryl-CoA hydrolase	F: GTGGAGGCGCGTCATAACGTC R: AGGAATGTGTCAGGGTCTT
Pparg2	peroxisome proliferator-activated receptor gamma 2	F: TTATAGCTGTCATTATTCTCAGTGGAG R: GACTCTGGGTGATTCAGCTTG
Rps13	ribosomal protein S13	F: CAGGTCCGTTTTGTGACTG R: AGCATCCTTATCCTTTCTGTT
Ucp1	uncoupling protein 1	F: GGGCATTCAGAGGCAAATCAG R: TTTCCGAGAGAAACAGGTGTTT
BCAT2	branched chain amino acid transaminase 2	F: CGCTCCTGTTCGTCATTCTCT R: CCCACCTAACTTGTAGTTGCC
BCKDHA	branched chain keto acid dehydrogenase E1, alpha polypeptide	F: CTACAAGAGCATGACACTGCTT R: CCCTCCTCACCATAGTTGGTC
HIBADH	3-hydroxyisobutyrate dehydrogenase	F: TGCTGCCCACCAGTATCAATG R: GCAGGATCAATAGTGCTGGAATC
HIBCH	3-hydroxyisobutyryl-CoA hydrolase	F: TGGTTCTTGCCAGAAACCTTATG R: GTAGCCACTCGAAATTGCCCA
HPRT1	hypoxanthine phosphoribosyltransferase 1	F: TGACCTTGATTTATTTTGCATACC R: CGAGCAAGACGTTCAGTCCT
IPO8	importin 8	F: CGGATTATAGTCTCTGACCATGTG R: TGTGTCACCATGTTCTTCAGG
PPARG2	peroxisome proliferator-activated receptor gamma 2	F: GAAAGCGATTCCTTCACTGAT R: TCAAAGGAGTGGGAGTGGTC

All primers used for qPCR were designed with Universal ProbeLibrary Assay Design Center from Roche. Primers used for mouse and human cells, respectively. Primer directions are indicated as F (Forward) and R (Reverse).

Supplementary Figures

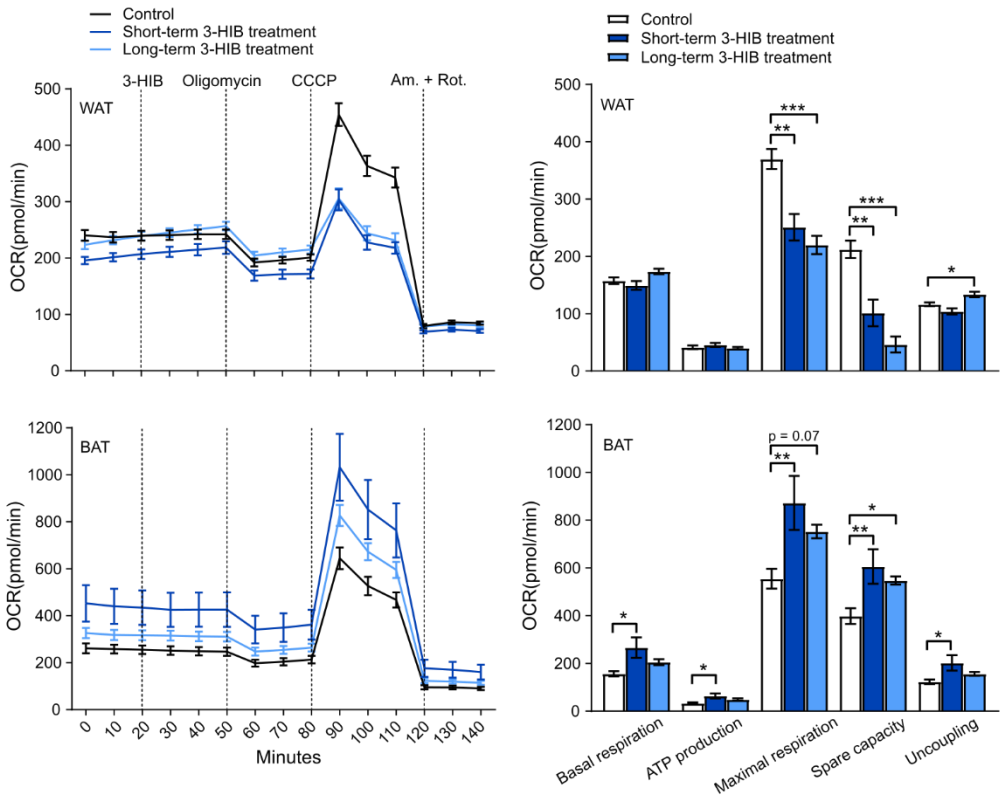


Supplementary Figure 1. Circulating 3-HIB is associated with type 2 diabetes and circulating BCAAs show dynamic changes after bariatric surgery.
(A) Plasma concentrations (adjusted geometric mean ± 95% CI) of 3-HIB and valine in normoglycemia (n = 4,537), hyperglycemia (n= 204) and diabetes groups without (n= 78) and with (n=123) diabetic drug use (metformin and/or insulin). The hyperglycemia group consisted of people with glucose values above 7 mmol/L but below 11.1 mmol/L. The diabetes group was based on self-reported diabetes with the addition of a few individuals with glucose > 11.1 mmol/L (HUSK cohort). (B) Serum concentrations of leucine and isoleucine in patients with obesity before and 1 year after bariatric surgery. Data are presented as mean ± SEM (t-test) (WNOB-1 cohort). (C) Individual serum concentrations of leucine and isoleucine in patients with obesity before, 1 week and 1 year after bariatric surgery (Roux-en-Y gastric bypass or laparoscopic sleeve gastrectomy) (WNOB-2 cohort). The black line indicates mean levels and grey lines individual levels. Pre, before bariatric surgery.
**P < 0.01, ***P < 0.001



Supplementary Figure 2. Levels of 3-HIB, BCAAs and BCAA catabolic enzyme expression in mouse and human adipocytes. (A-B) Extracellular (cell culture medium, 48 h consumption) levels of the metabolite 3-HIB and valine, leucine, isoleucine (BCAAs) during differentiation of 3T3-L1 (n=3). (C, D) Extracellular (cell culture

medium, 48 h consumption) and intracellular (total cell lysate) levels of the metabolite 3-HIB and valine, leucine, isoleucine (BCAAs) of undifferentiated and differentiated (C) 3T3-L1 adipocytes (per 10x6 cells) and (D) immortalized human white adipocytes (normalized to protein concentration) during differentiation. (n=1 per timepoint, samples originating from six 15-cm dishes pooled together). (E, F) Representative images of oil red o lipid stained cells (left) and quantification of lipid accumulation (right) (n=3) of (E) 3T3-L1 and (F) human white adipose tissue cells (immortalized). (G, H) Relative mRNA levels of genes encoding BCAA catabolic enzymes during adipogenic differentiation in (G) primary human adipocytes (n=9-12, pooled data for cultures from donors 3-6) and (H) immortalized hWAT day 0 (pre-diff) and day 12 (n=3-4). Statistical significance was calculated compared to day 0. The results are presented as mean $\pm$ SEM. (I) Representative western blot, showing protein levels of HIBCH and (J) the quantitative values of HIBCH protein relative to α-VCL (α-VINCULIN) (n=3; relative values of 3 independent experiments each day of differentiation) and *Hibch* mRNA level (n=3) in 3T3-L1 during differentiation. (K) Extracellular (cell culture medium, 48 h consumption) and intracellular levels of the BCAAs (leucine, isoleucine and valine) relative to protein content in undifferentiated controls and cells differentiated for 8 days (n=1 per timepoint, samples originating from six 15-cm dishes pooled together).
*P < 0.05, **P < 0.01, ***P < 0.001



Supplementary Figure 3. 3-HIB affects mitochondrial respiration in WAT and BAT adipocytes. Seahorse Cell Mito Stress Assay (OCR measurements) was performed using the Seahorse XFe96 Analyzer to assess the mitochondrial respiration in WAT and BAT (n=6-15) at day 6 in differentiation. 3-HIB treatment (final concentration of 10 mM) was added for 48 hours from day 4 to 6 (long-term treatment) or directly in the Seahorse XFe96 Analyzer after the three first OCR measurements (day 6, short-term treatment), before adding oligomycin, CCCP and rotenone/antimycin A, as indicated at the top in the upper left figure. Outliers were removed based on a Whisker Tukey test of the OCR data for each timepoint in each well. Basal respiration, ATP production, maximal respiration, spare capacity and uncoupling were calculated for each well based on the OCR measurements. Data were normalized to cell counts per well by Hoechst staining. One-way ANOVA with Dunnett's post-test was used for multiple comparisons of treatments relative to control.
*P < 0.05, **P < 0.01, ***P < 0.001