Supplemental Data

# Genetic disruption of uncoupling protein 1 (UCP1) in mice renders brown adipose tissue a significant source of FGF21 secretion

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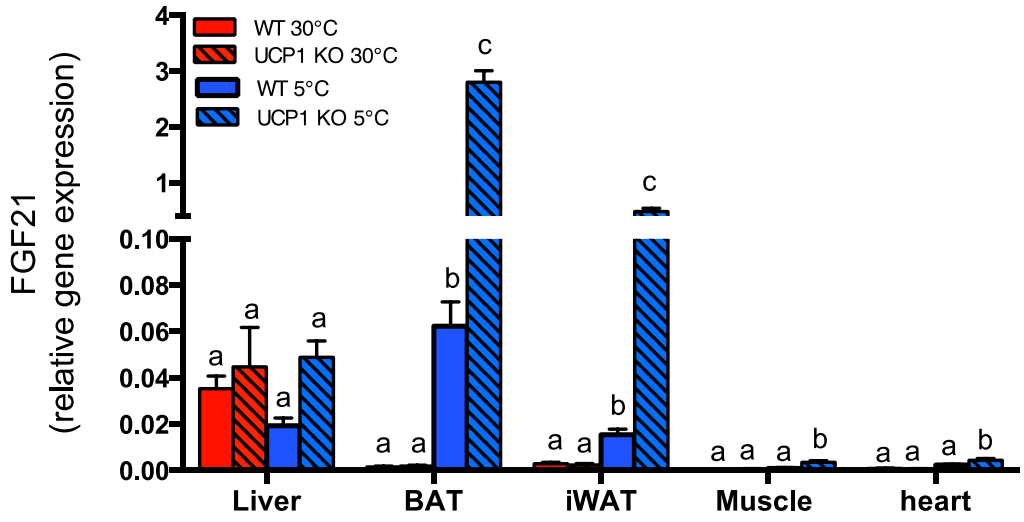
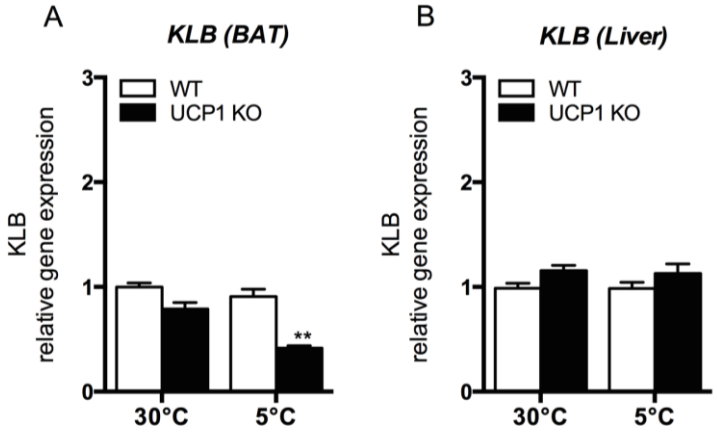
***Supplementary Figure S1. The Comparative qPCR Analysis of FGF21 in Liver, BAT, iWAT, Skeletal Muscle and Heart***

To identify tissues that potentially contribute to increasing FGF21 serum levels of UCP1 KO mice under cold conditions, we determined FGF21 mRNA levels in liver, skeletal muscle, heart, white and brown adipose tissue (Fig. S1). In contrast to the main figure, gene expression was calculated considering ct-values without normalizing to tissue-specific housekeeping genes, which allows multi-tissue comparison.



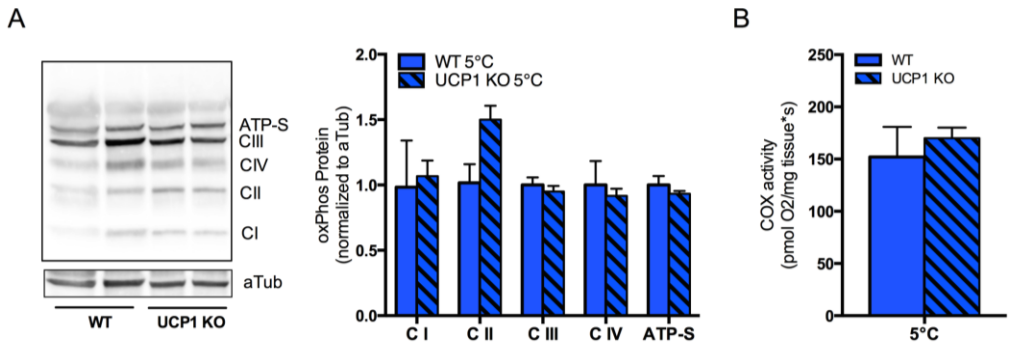

***Supplementary Figure S2. Beta Klotho (KLB) Gene Expression In BAT and Liver of WT and UCP1 KO Mice***

The biological activity of circulating FGF21 depends on its binding to FGF receptors and the presence of co-receptor beta-Klotho (KLB), which is crucial for FGF21 specificity and metabolic action [1, 2]. KLB gene expression was significantly increased in iWAT (Figure 3D), but not BAT or liver, of cold acclimated UCP1 KO mice, suggesting that this tissue is a potential target for circulating FGF21 (Fig. S2A-B).



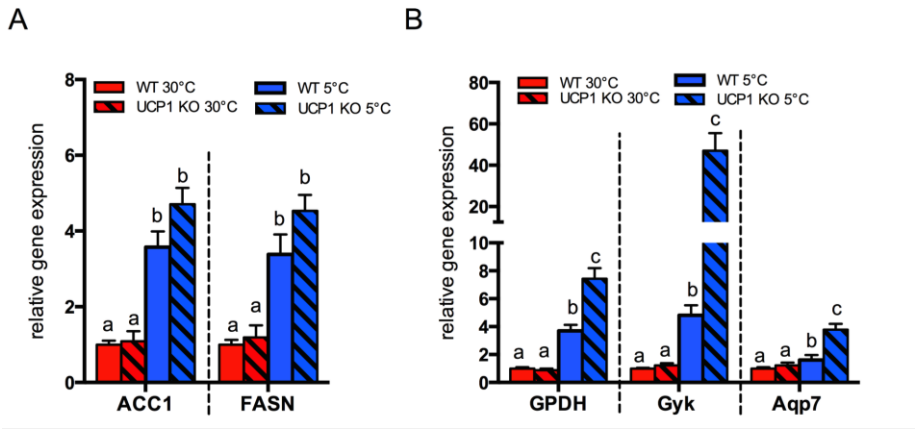
***Supplementary Figure S3.*** ***No differences in oxidative capacity in iWAT of cold adapted UCP1 KO mice.***

Mice were exposed to 5°C for 3 weeks (upon acclimation to 18°C for 2 weeks). (A) Representative image of Western Blot and relative quantification of proteins of the respiratory chain (OXPHOS Rodent WB Antibody Cocktail contains five antibodies, one each against Complex 1- CI subunit NDUFB8, Complex 2 - CII, Complex 3- CIII Core protein 2, Complex 4 - CIV subunit I and ATP-Synthase - ATP-S alpha subunit). (B) Cytochrome-c Oxidase (Cox) activity were measured in UCP1 KO mice and WT littermates kept 3 weeks at 5°C.



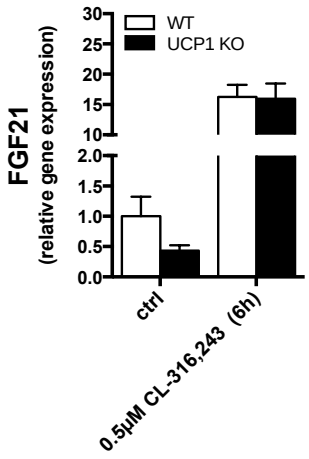

***Supplementary Figure S4. Gene expression of Lipid and Glycerol Metabolism in iWAT of UCP1 KO Mice***

Lipid metabolites in blood serum were changed. Therefore, we investigated expression of lipolytic and lipogenic genes. The expression of the lipolytic gene ATGL was significantly increased (Fig. 3G), and lipogenic genes (ACC, FASN) trended toward higher levels in UCP1 KO mice (Fig. S4A), suggesting futile cycling of fatty acid metabolism. The increased futile cycling of triglyceride hydrolysis and re-synthesis are promoted by the induction of glycerol-kinase during cold exposure in WAT [3], prompting us to measure the gene expression of glycerolkinase (Gyk) and the adipocyte glycerol transporter Aqp7. Gyk and Aqp7 showed enhanced cold induction in UCP1 KO mice as compared to WT mice (Fig. S4B). These results are suggestive for futile cycling of triglycerides in iWAT that may be mediated by circulating FGF21.




***Supplementary Figure S5. No Quantitative Genotype Differences in FGF21 Gene Expression of Primary Brown Adipocytes after Beta3-adrenergic Agonism using CL 316,243***

To investigate if the control for the increased brown adipose tissue FGF21 release is extrinsic (e.g. sympathetic nervous over-activation) or intrinsic by direct effects of UCP1 ablation, we treated primary brown adipocytes of WT and UCP1 KO mice with the beta3-adrenergic agonist CL 316,243. We show that there are no genotype differences between WT and UCP1 KO mice. Thus, the *in vivo* phenotype of high FGF21 mRNA expression is likely extrinsic (Fig. S5).



## Supplementary Methods

### *Primary brown adipocytes*

The isolation of the stromal vascular (SV) fraction from the interscapular brown fat (iBAT) pad of WT and UCP1 KO mice (six weeks old) was performed as follows. iBAT fat pads were minced and then digested for 40min at 37°C in digestion buffer (PBS, 3U/ml Dispase II, 0.01mM CaCl, 1 mg/mL collagenase II). Digested tissue was then filtered through a 100-µm cell strainer and centrifuged at 500g for 10min at 4°C. The cell pellet was resuspended in growth medium (DMEM/F12, 10% FBS, 1% P/S), filtered through a 70-µm cell strainer, centrifuged at 500g for 10min at 4°C and resuspended in growth media. SV cells were plated onto in 12-well plates allowing them to grow to 90–100% confluence. At confluence, differentiation was started using induction media (for 2 days; Growth Media, 5µM Dexamethasone, 0.5mM IBMX, 125µM Indomethacine, 1µM Rosiglitazone, 0.5µg/ml Insulin, 1nM T3) followed with contiuation media (for 2 days; Growth Media, 1µM Rosiglitazone, 0.5µg/ml Insulin, 1nM T3) and differentiation media (for 2 days; Growth Media, 0.5µg/ml Insulin, 1nM T3). On day 6 of differentiation, half of the cells were treated with 0.5µM CL 316,243 for 6h. At the end of the experiment, cells were harvested for RNA isolation.

### *Immunological detection*

For protein extraction, tissue was homogenized in RIPA buffer (150 mM NaCl, 50 mM TRIS, 0.1% SDS, 1% IGEPAL CA-630, 0.5% Sodium deoxycholate; pH 7.4-7.6) containing protease inhibitor cocktail (Halt ™ Protease & Phosphatase Inhibitor Cocktail, Thermo Scientific) and protein concentration was measured using Bradford reagent (Sigma). The following primary antibodies were used: UCP1 (rabbit anti-hamster UCP1), GAPDH (sc-166545; Santa Cruz Biotechnology) and total OXPHOS Rodent WB Antibody Cocktail (ab110413, Abcam). Horseradish-peroxidase-conjugated secondary antibodies were used: anti-rabbit IgG (sc-2004, Santa Cruz Biotechnology) or anti-mouse IgG (sc-2005, Santa Cruz Biotechnology).

## Supplementary References

**1.** Adams, A.C., Cheng, C.C., Coskun, T., and Kharitonenkov, A. (2012). FGF21 requires betaklotho to act in vivo. Plos One *7*, e49977.

2. Ding, X., Boney-Montoya, J., Owen, B.M., Bookout, A.L., Coate, K.C., Mangelsdorf, D.J., and Kliewer, S.A. (2012). betaKlotho is required for fibroblast growth factor 21 effects on growth and metabolism. Cell Metab *16*, 387-393.

3. Rosell, M., Kaforou, M., Frontini, A., Okolo, A., Chan, Y.W., Nikolopoulou, E., Millership, S., Fenech, M.E., MacIntyre, D., Turner, J.O.*, et al.* (2014). Brown and white adipose tissues: intrinsic differences in gene expression and response to cold exposure in mice. American journal of physiology Endocrinology and metabolism *306*, E945-964.