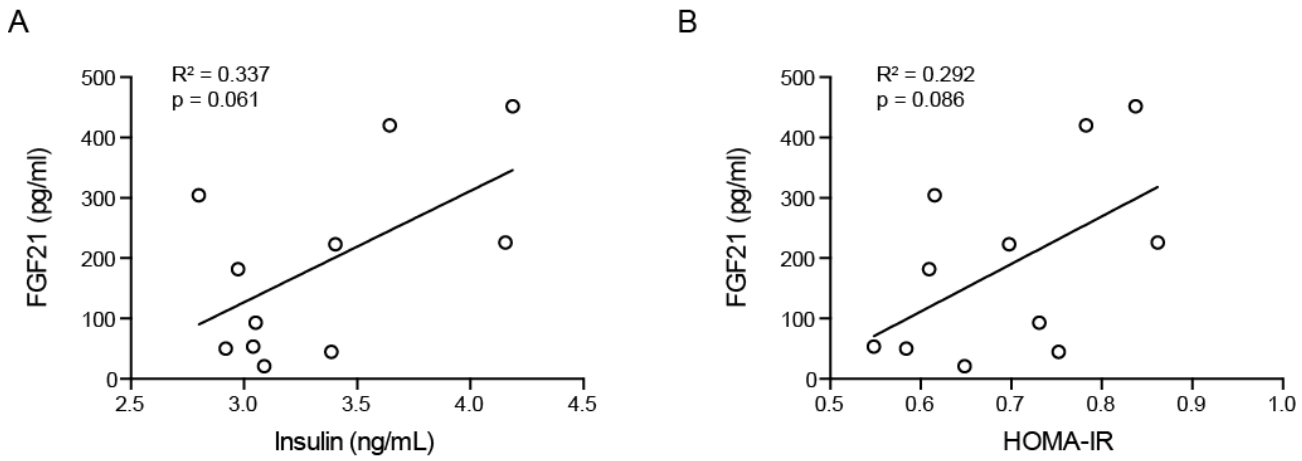
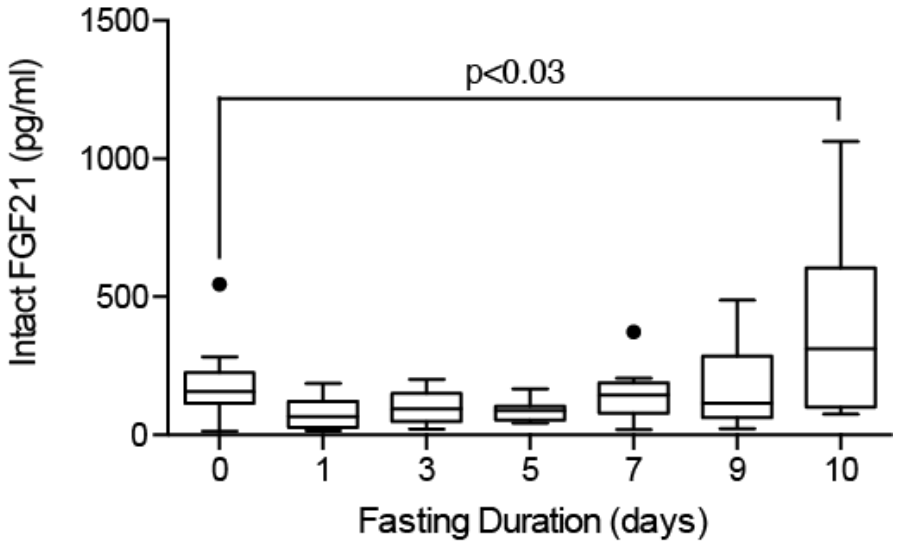


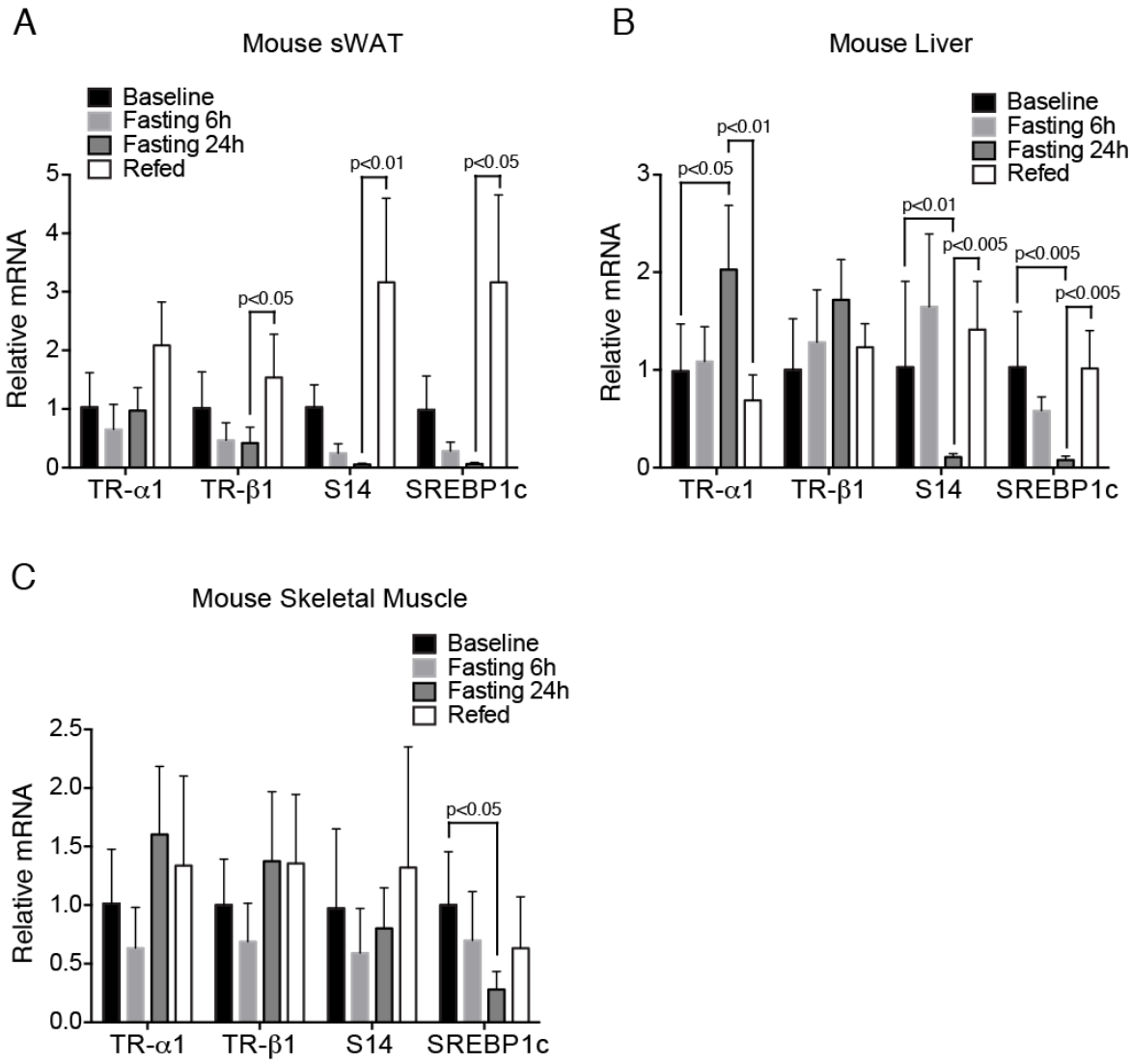
Supplemental Figure 1. Circulating FGF21 and measures of insulin sensitivity in humans.

A) Linear correlation between baseline circulating FGF21 levels and baseline serum insulin levels; analysis performed using least squares regression.

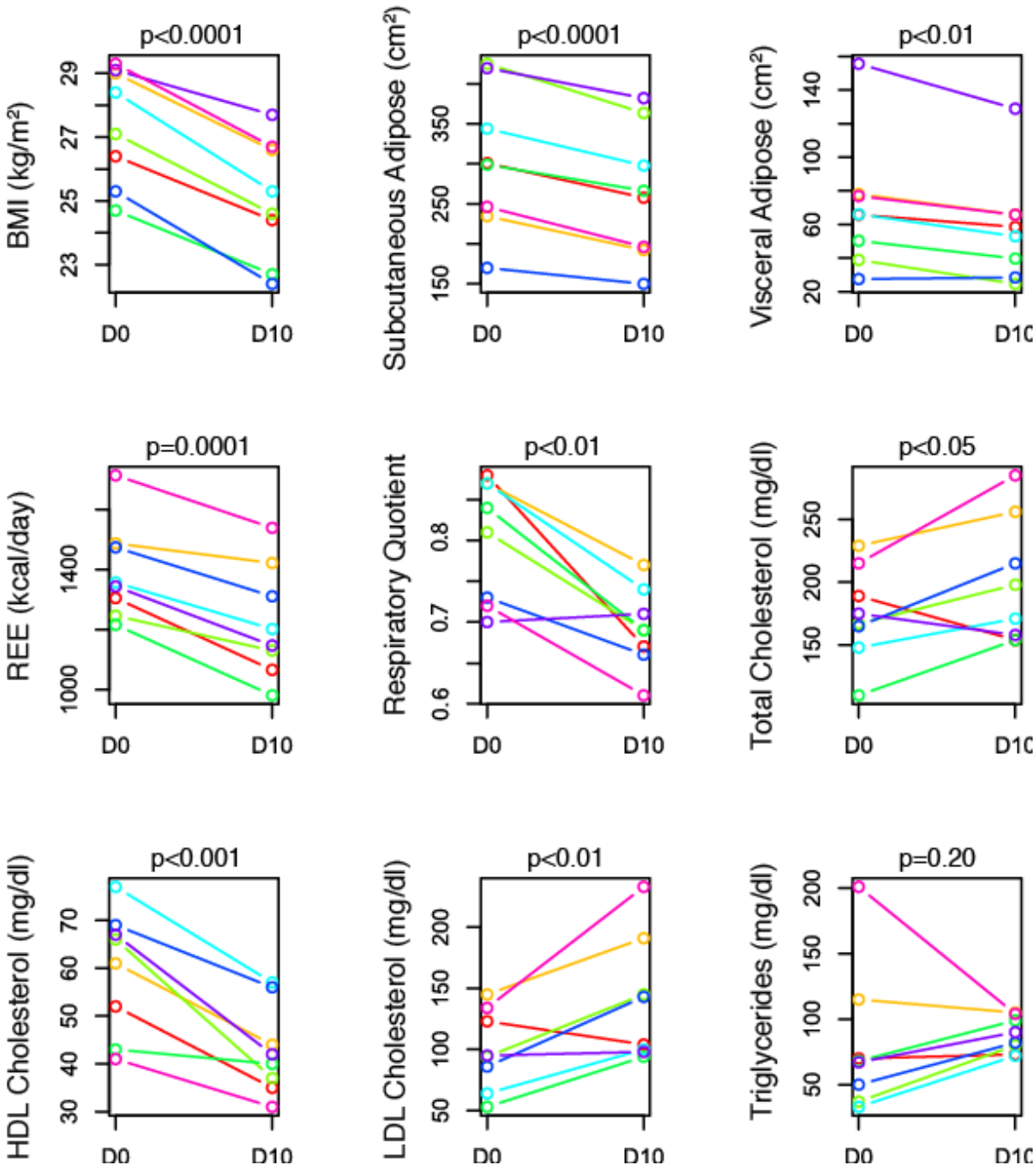
B) Linear correlation between baseline circulating FGF21 levels and the homeostatic model assessment for insulin resistance (HOMA-IR); analysis performed using least squares regression.



Supplemental Figure 2. Intact FGF21 is induced by prolonged fasting in humans. Human samples measured and displayed in Figure 1 were reanalyzed using an ELISA assay specific for the full-length intact FGF21 protein and displayed using a Tukey box plot. Baseline and day 10 intact FGF21 levels were compared using the Wilcoxon signed-rank test.



Supplemental Figure 3. Thyroid pathway transcriptional activity during fasting and refeeding in mice. C57Bl6 mice were fasted for 6 or 24 hours and then refed for 6 hours (n=5 per timepoint, statistical significance assigned for $p<0.05$, ANOVA followed by Sidak's correction for multiple comparisons). The analysis was conducted for (A) mouse subcutaneous adipose tissue (sWAT), (B) mouse liver, and (C) mouse skeletal muscle.



Supplemental Figure 4. Pre- and post-fast measurements of metabolic function and body composition. Measurements were performed at baseline (D0) and after 10 days of fasting (D10). Variables were compared with a paired t-test; for non-normally distributed data (visceral adipose tissue, respiratory quotient and triglycerides), a Wilcoxon signed-rank test was used. The subject who achieved a respiratory quotient of 0.61 was the same

subject (and only subject) who did not mount an FGF21 response to fasting (baseline FGF21 level: 452 pg/ml and final fast day FGF21 level: 230 pg/ml).

	r	95% CI	Adjusted P-value
Resting Energy Expenditure	-0.72	(-0.95, -0.04)	0.35
O2 Ventilation Rate	-0.62	(-0.92, 0.15)	0.7
LDL cholesterol	-0.58	(-0.91, 0.21)	0.8
Triglycerides	0.52	(-0.29, 0.90)	0.94
Total cholesterol	-0.38	(-0.86, 0.44)	1
HDL cholesterol	0.32	(-0.49, 0.84)	1
Subcutaneous Adipose Tissue	0.73	(0.06, 0.95)	0.35
Visceral Adipose Tissue	0.31	(-0.5, 0.83)	1
Body Mass Index	0.27	(-0.54, 0.82)	1
T3	-0.45	(-0.88, 0.38)	1

Supplemental Table 1. Correlation of Change in Metabolic Predictors with Day 10 FGF21 serum levels. The difference between Day 10 and Day 0 observations for each predictor were analyzed for correlation with log-transformed FGF21 at Day 10. Pearson's r was calculated and reported with 95% confidence intervals (95% CI). P-values are adjusted for multiple analyses.

Human		
Gene	Direction	Sequence
βKlotho	F	TTCTGGGGTATTGGGACTGGA
	R	CCATTCGTGCTGCTGACATTTT
Cidea	F	GATGCCCTCGTCATCGCTAC
	R	GCGTGTTGTCTCCCAAGGTC
DIO2	F	TCCAGTGTGGTGCATGTCTC
	R	CTGGCTCGTGAAAGGAGGTC
FGFR1	F	GGCTACAAGGTCCGTTATGCC
	R	GATGCTGCCGTACTCATTCTC
GLUT1	F	ATTGGCTCCGGTATCGTCAAC
	R	GCTCAGATAGGACATCCAGGGTA
PGC1α	F	TCTGAGTCTGTATGGAGTGACAT
	R	TCTGAGTCTGTATGGAGTGACAT
PPARα	F	ATGGTGGACACGGAAAGCC
	R	CGATGGATTGCGAAATCTCTTGG
PPARγ	F	TACTGTCGGTTTCAGAAATGCC
	R	GTCAGCGGACTCTGGATTCAG
PRDM16	F	CTTCGGATGGGAGCAAATACTG
	R	TCCACGCAGAACTTCTCACTG
S14	F	CCAAGAACTGCCTGCTGACCGTCATGG
	R	GGATGTGATGGAGGCTGGAGAAGTGC
SREBP1c	F	CCATGGATTGCACTTTCGAA
	R	GGCCAGGGAAGTCACTGTCTT
TR-α1	F	AGGTCACCAGATGGAAAGCG
	R	AGTGATAACCAGTTGCCTTGTC
TR-β1	F	CCAGAAGACATTGGACAAGCA
	R	GCAGCTCACAAAACATAGGCA
UCP1	F	CAATCACCGCTGTGGTAAAAAC
	R	GTAGAGGCCGATCCTGAGAGA

Mouse		
βKlotho	F	TGTTCTGCTGCGAGCTGTTAC
	R	CCGGACTCACGTACTGTTTTT
FGF21	F	CTGCTGGGGGTCTACCAAG
	R	CTGCGCCTACCACTGTTCC
FGFR1	F	TAATACCACCGACAAGGAAATGG
	R	TGATGGGAGAGTCCGATAGAGT
GLUT1	F	TCAACACGGCCTTCACTG
	R	CACGATGCTCAGATAGGACATC
PPARα	F	AGAGCCCCATCTGTCCTCTC
	R	ACTGGTAGTCTGCAAAACCAAA
PPARγ	F	TCGCTGATGCACTGCCTATG
	R	GAGAGGTCCACAGAGCTGATT
S14	F	ATGCAAGTGCTAACGAAACGC
	R	CCTGCCATTCCTCCCTTGG

SREBP1c	F	GGAGCCATGGATTGCACATT
	R	GCTTCCAGAGAGGAGGCCAG
TR-α1	F	TGCCTTTAACCTGGATGACAC
	R	TCGACTTTCATGTGGAGGAAG
TR-β1	F	AGCCAGAACCCACGGATGAGGA
	R	TGCCACCTTCTGGGGCATTCAC
S14	F	CAATCACCGCTGTGGTAAAAAC

Supplemental Table 2. qPCR primers. For analysis of human FGF21 transcription, a previously validated Taqman primer set was utilized (Life Technologies, Hs00173927_m1).