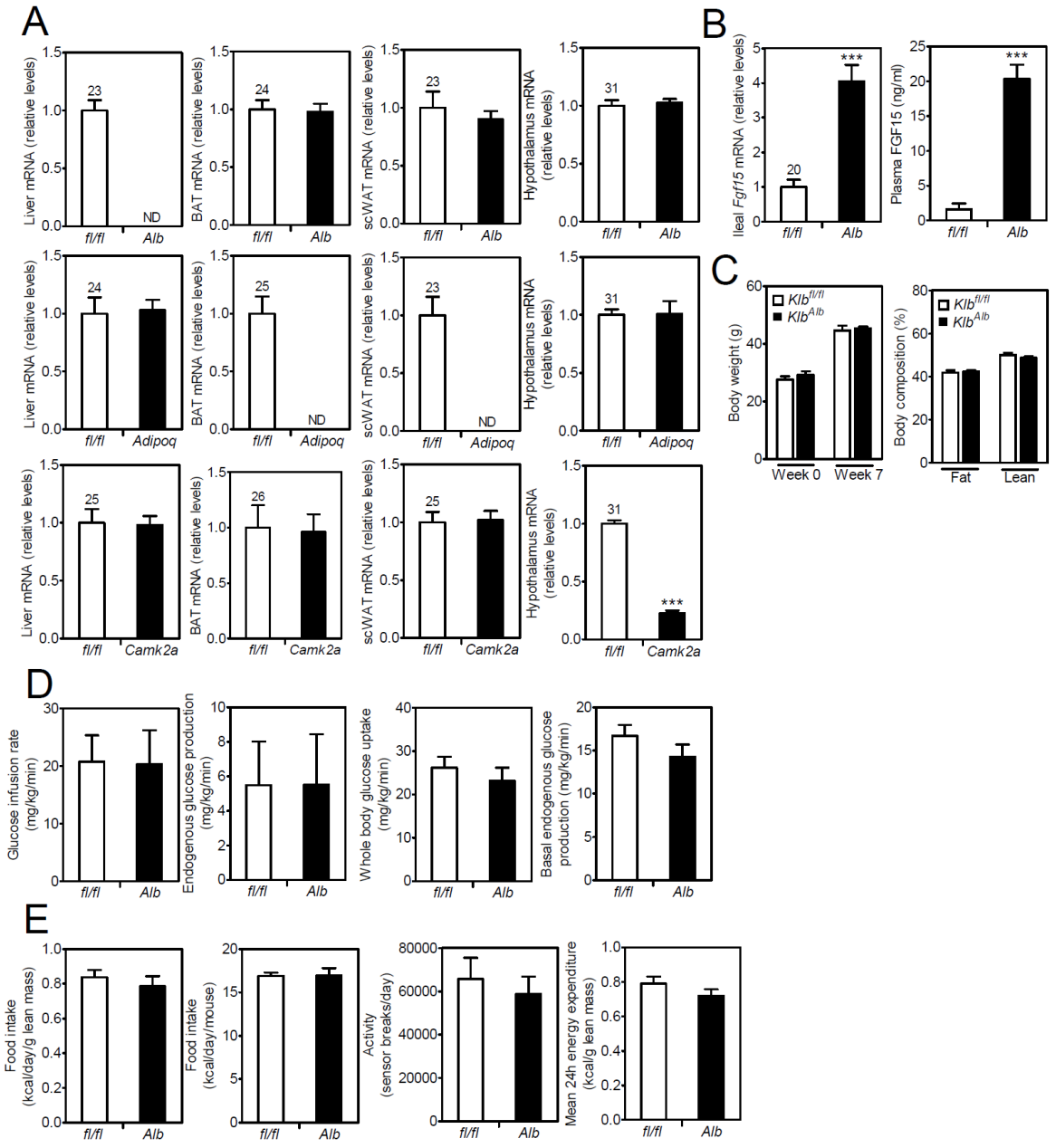

Figure S1, related to Figure 1, Figure 2, Figure 4 and Figure 5. β-Klotho expression in tissue-specific β-Klotho knockout mouse models and metabolic comparison between *Klb*$^{fl/fl}$ and *Klb*^{Alb} mice.

(A) QPCR analysis of β-Klotho mRNA levels in liver, BAT, scWAT and hypothalamus in DIO *Klb*^{Alb} mice, *Klb*^{Adipoq} mice, *Klb*^{Camk2a} mice and their corresponding DIO *Klb*$^{fl/fl}$ littermates. QPCR cycle time values are shown for the *Klb*$^{fl/fl}$ group. n = 5-6/group.

(B) Ileal *Fgf15* mRNA levels and plasma FGF15 protein levels in DIO *Klb*$^{fl/fl}$ and *Klb*^{Alb} littermates. QPCR cycle time values are shown for the *Klb*$^{fl/fl}$ group. n = 5-7/group.

(C) Body weight in chow-fed lean and DIO *Klb*$^{fl/fl}$ and *Klb*^{Alb} littermates after 7 weeks on high-fat diet (left panel). Body composition in DIO *Klb*$^{fl/fl}$ and *Klb*^{Alb} littermates after 7 weeks on high-fat diet (right panel). n = 8/group.

(D) Hyperinsulinemic-euglycemic clamp was performed in DIO *Klb*$^{fl/fl}$ and *Klb*^{Alb} littermates. Glucose infusion rate, endogenous glucose production and whole-body glucose uptake during the clamp and basal endogenous glucose production measured. n = 5/group.

(E) 24hr food intake, activity and energy expenditure were measured in DIO *Klb*$^{fl/fl}$ and *Klb*^{Alb} littermates. n = 8/group.

Data are shown as the mean ± SEM. *p < 0.05, **p < 0.01, ***p<0.001 compared to control. ND, not detected.

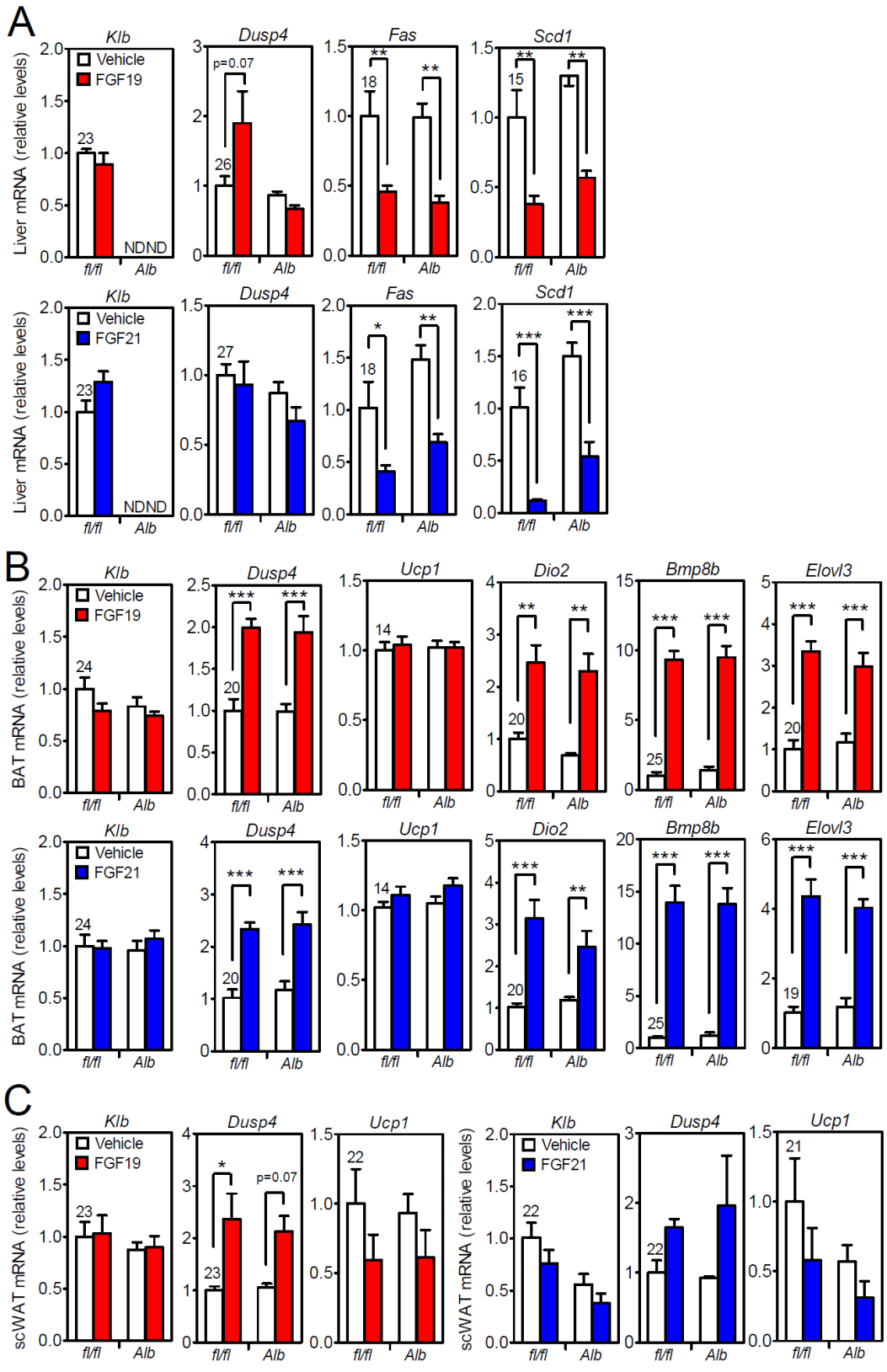

Figure S2, related to Figure 2. Gene expression in control and liver-specific β-Klotho knockout mice.
(A-C) QPCR analysis of gene expression in (A) liver, (B) BAT and (C) scWAT. QPCR cycle time values are shown for the vehicle-treated $Klb^{fl/fl}$ group.
Data are shown as the mean ± SEM. *p < 0.05, **p < 0.01, ***p<0.001 compared to control. ND, not detected.

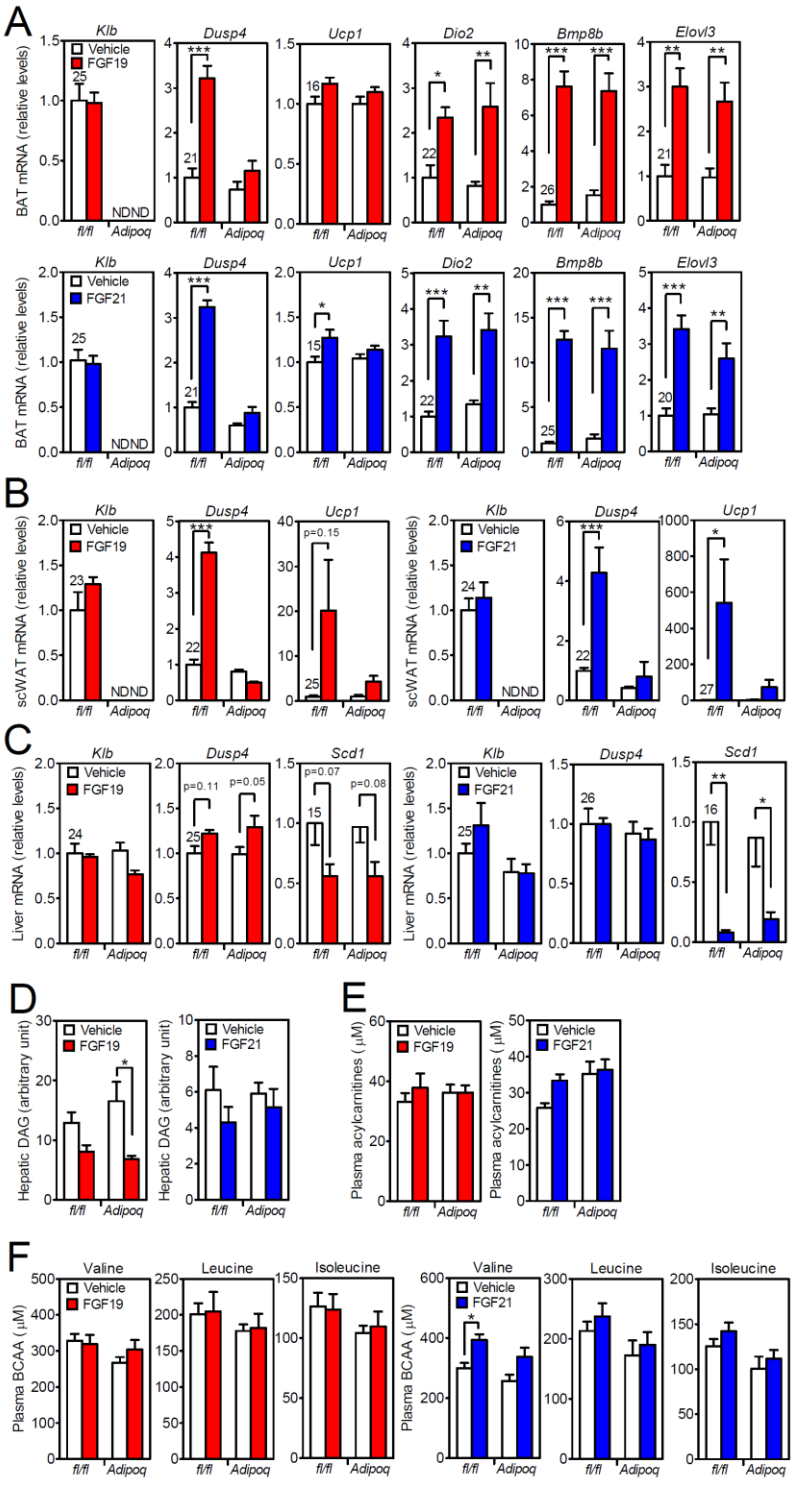

Figure S3, related to Figure 4. Gene expression, hepatic diacylglycerol levels and plasma acylcarnitines and branched-chain amino acid levels in control and adipose tissue-specific β-Klotho knockout mice.

(A-C) QPCR analysis of gene expression in (A) BAT, (B) scWAT and (C) liver. QPCR cycle time values are shown for the vehicle-treated $Klb^{fl/fl}$ group.

(D) Hepatic diacylglycerol (DAG) levels.

(E) Plasma acylcarnitine levels.

(F) Plasma branched-chain amino acid (BCAA) levels.

Data are shown as the mean ± SEM. *p < 0.05, **p < 0.01, ***p<0.001 compared to control. ND, not detected.

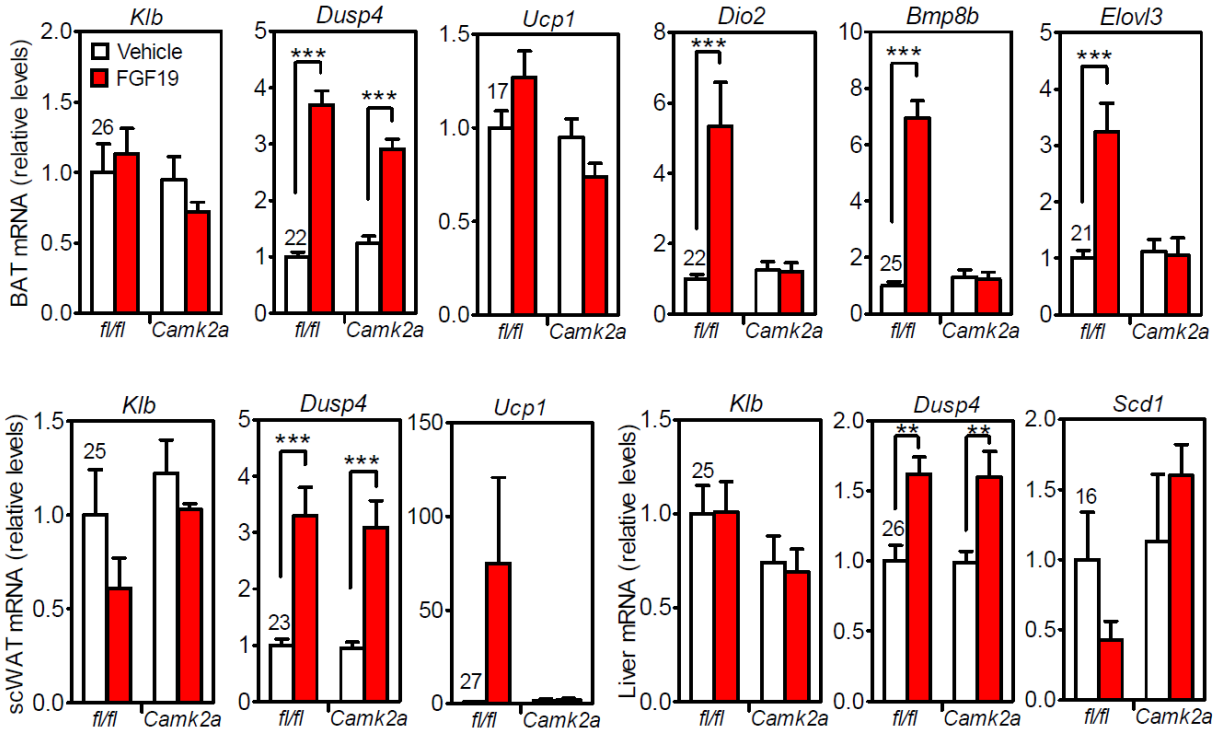

Figure S4, related to Figure 5. Gene expression in control and nervous system-specific β-Klotho knockout mice. QPCR analysis of gene expression in BAT, scWAT and liver. QPCR cycle time values are shown for the vehicle-treated *Klb*$^{fl/fl}$ group.
Data are shown as the mean ± SEM. *p < 0.05, **p < 0.01, ***p<0.001 compared to control.

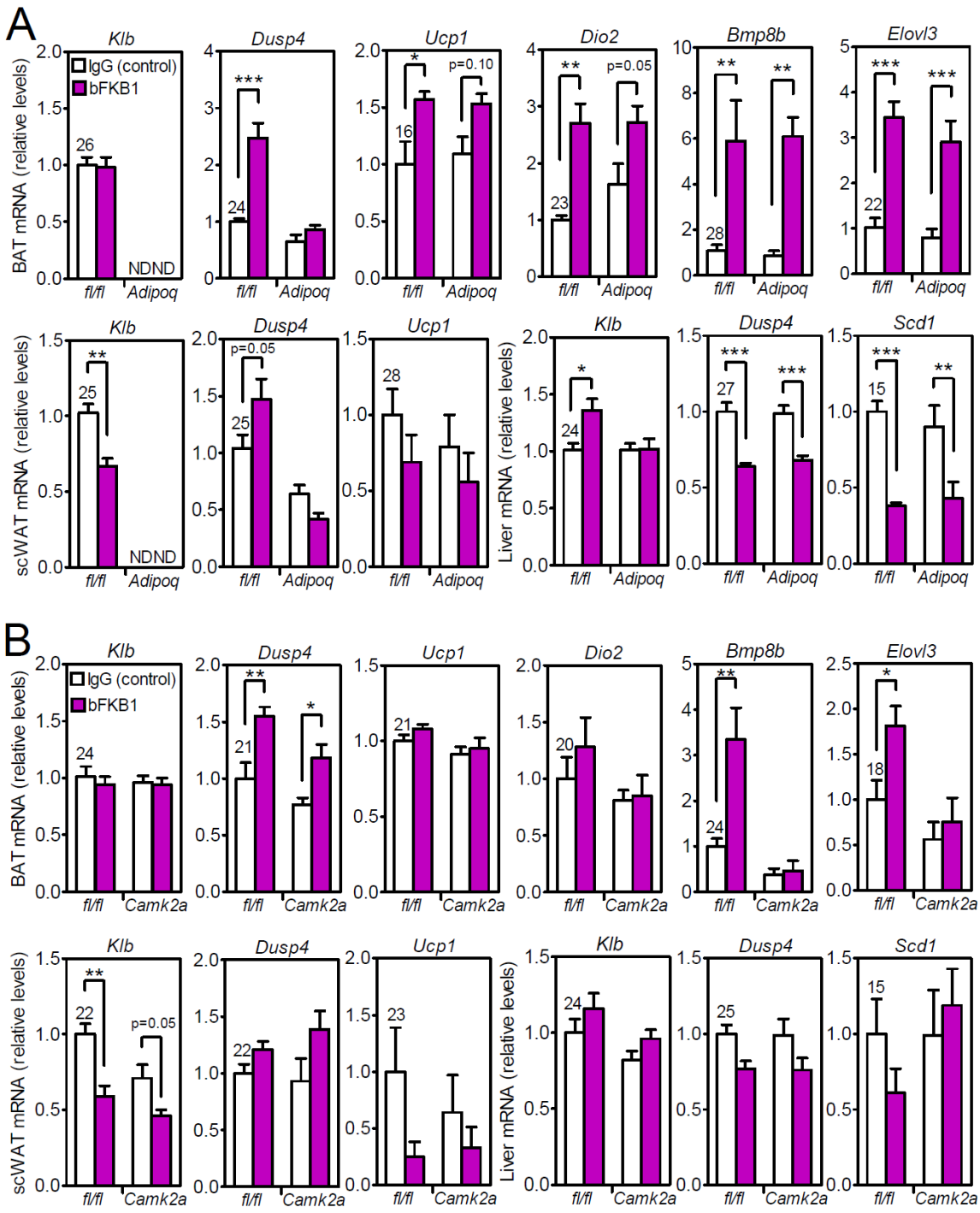

Figure S5, related to Figure 6. Gene expression in mice treated with the FGFR1c/β-Klotho activating antibody.

(A) QPCR analysis of gene expression in BAT, scWAT and liver in DIO $Klb^{fl/fl}$ and Klb^{Adipoq} littermates administered either bFKB1 or control antibody (trastuzumab) for 4 wk. QPCR cycle time values are shown for the control antibody-treated $Klb^{fl/fl}$ group.

(B) QPCR analysis of gene expression in BAT, scWAT and liver in DIO $Klb^{fl/fl}$ and Klb^{Camk2a} littermates administered either bFKB1 or control antibody (trastuzumab) for 4 wk. QPCR cycle time values are shown for the control antibody-treated $Klb^{fl/fl}$ group.

Data are shown as the mean ± SEM. *p < 0.05, **p < 0.01, ***p<0.001 compared to control. ND, not detected.

Table S1, related to Figure S1-S5. Summary of primers used for QPCR analyses.

Gene Name	Primer Sequences
U36B4	Forward 5'cgtcctcgttggagtgaca3' Reverse 5'cggtgcgtcagggattg3'
Bmp8b	Forward 5'accaaccacgccactatgc3' Reverse 5'cagtaggcacacagcacacctt3'
Cyp7a1	Forward 5'agcaactaaacaacctgccagtacta3' Reverse 5'gtccggatattcaaggatgca3'
Dio2	Forward 5'gtccgcaaatgacccctttt3' Reverse 5'cccacccactctctgactttc3'
Dusp4	Forward 5'accacaaggccgacatcag3' Reverse 5'gtcctttactgcgtcgatgtactc3'
Egr1	Forward 5'cgagcgaacaaccctatgag3' Reverse 5'cattattcagagcgatgtcagaaa3'
Elovl3	Forward 5'cttcgagacgtttcaggacttaag3' Reverse 5'tctggccaacaacgatgag3'
Fas	Forward 5'gctgcggaaacttcaggaaat3' Reverse 5'agagacgtgtcactcctggactt3'
Fgf15	Forward 5'acgggctgattcgctactc3' Reverse 5'tgtagcctaaacagtccatttcct3'
Klb	Forward 5'gatgaagaatttcctaaaccaggtt3' Reverse 5' aaccaaacacgcggatttc3'
Scd1	Forward 5'tgcccctgcggatctt3' Reverse 5'gcccattcgtacacgtcatt3'
Ucp1	Forward 5'aagctgtgcgatgtccatgt3' Reverse 5'aagccacaaaccctttgaaaa3'