SUPPLEMENTARY DATA

Justification

In the study we demonstrate that circulating FGF21 is dramatically increased in glucagon receptor deficient mice. In order to understand the regulation of this process we looked for metabolic indicators that may correlate with FGF21 levels. We found that FGF21 levels correlated with fasting glucose. As the focus of the article is on the action of FGF21 we felt that this information does not warrant a figure in the main document. Our focus in this study was the role of FGF21 in the insulin and glucagon independant regulation of glucose metabolism *in vivo* in mice. To do this, we make use of a neutralizing antibody. Although we show that it effectively neutralizes in vivo, we also want to show that it neutralizes FGF21 downstream signaling *in vitro*. This technical validation is not a result that warrants demonstration in the main document but we feel it provides valuable information.

Supplementary Table 1.

Correlation FGF-21/basal glucose	**Spearman r**	**p-value**
Gcgr$^{+/+}$	-0.4506	0.0462
Gcgr$^{-/-}$	-0.8451	0.0008

Supplementary Figure 1. HEK293 cells overexpressing Beta-Klotho were cultured in 96-well plates. The activity of FGF21 was measured by phosphorylation of the downstream target ERK. In brief, cells were incubated for 12 minutes with FGF21 (closed circles) or FGF21 incubated with increasing amounts, 1:1000 (closed triangles); 1:200 (closed squares), of neutralizing antibody overnight. Cells were harvested and phosphorylated ERK was measured using the AlphaScreen SureFire Assay (Perkin Elmer, U.S.A.).