

Generation of a miniature pig disease model for human Laron syndrome

Dan Cui, Fang Li, Qiuyan Li, Jia Li, Yaofeng Zhao, Xiaoxiang Hu, Ran Zhang^{*},
Ning Li^{*}

State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing
100193, China

Corresponding author: Ning Li and Ran Zhang

State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing
100193, China

Tel: +86 10 6273 3323; Fax: +86 10 6273 3904

^{*}E-mail: ninglcau@cau.edu.cn; zhangran0628@cau.edu.cn

Supplementary Materials

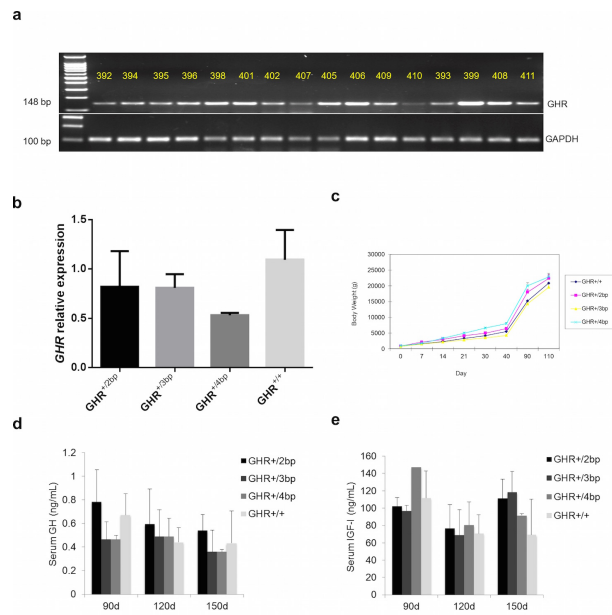
Supplementary Figure S1. Identification of F0 heterozygous pigs

Supplementary Figure S2. Identification of F1 $GHR^{+/4bp}$ pigs by T7E1 cleavage

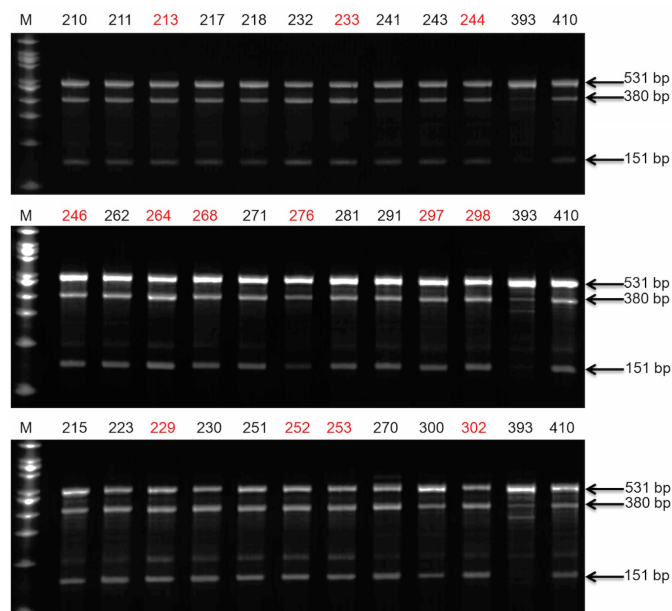
Supplementary Figure S3. Identification of *GHR* mRNA expression in the liver tissues of F2 pigs

Supplementary Figure S4. GHBP levels in the F2 generation

Supplementary Figure S5. Reduced bone growth in $-/-$ pigs

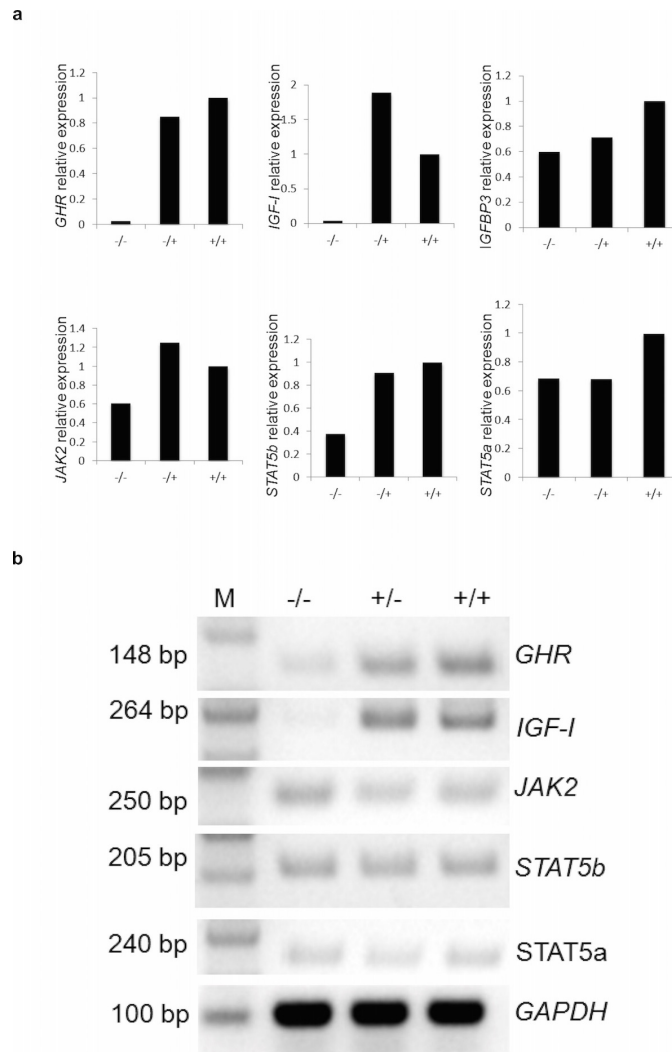


Supplementary Figure S1 | Identification of F0 heterozygous pigs. Detection and quantification of *GHR* mRNA expression in F0 pigs by RT-PCR (a) and qRT-PCR (b). Numbers indicate individual F0 pigs; $GHR^{+/4bp}$, $GHR^{+/2bp}$, $GHR^{+/3bp}$ are the different genotypes in the F0 heterozygous pigs. The expression levels were determined by the expression relative to *GAPDH* (an internal control). The data were combined from three independent experiments; the bars represent the means \pm SD. (c) No significant difference in body weight was seen between F0 heterozygous pigs and wild-type controls. Neither a significantly increased level of GH (d) nor a significantly reduced level of IGF-I (e) was observed in F0 heterozygous pigs compared with the wild-type controls. The bars represent the means \pm SD.



Supplementary Figure S2 | Identification of F1 GHR^{+4bp} pigs by T7E1 cleavage.

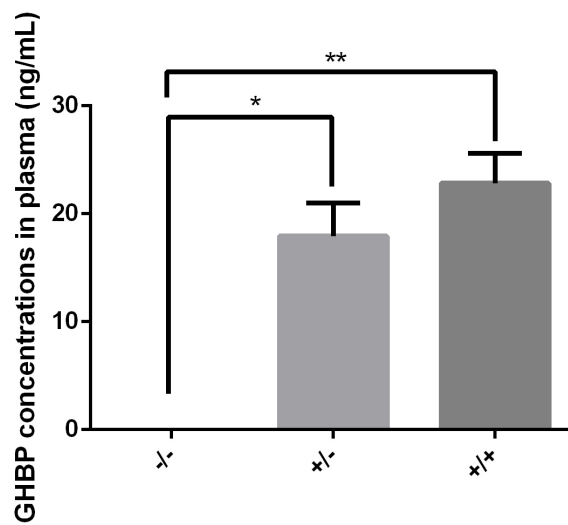
The products after cleavage were separated on 2.5% agarose; the expected sizes are 380 bp and 151 bp after T7E1 cleavage; all the numbers represent the positive GHR^{+4bp} pigs in the F1 generation; red numbers indicate the female GHR^{+4bp} pigs.



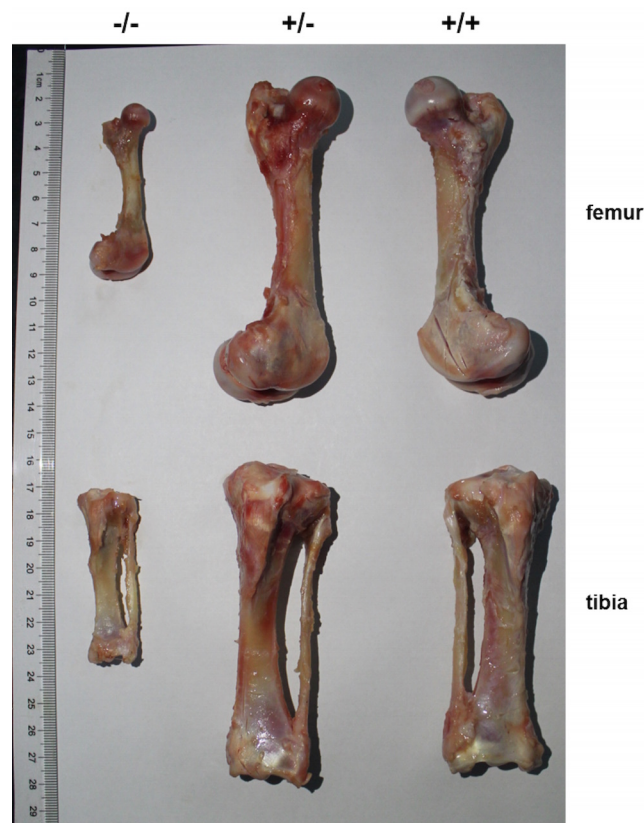
Supplementary Figure S3 | Identification of *GHR* mRNA expression in the liver

tissues of F2 pigs. (a) Quantification of *GHR*, *IGF-I*, *IGFBP3*, *JAK2*, *STAT5b* and *STAT5a* mRNA expression in the liver tissues of F2 pigs by qRT-PCR. The expression levels were determined by the expression relative to *GAPDH* (an internal control); the data were combined from three independent experiments, with one pig

per group. (b) RT-PCR results of the liver expression of *GHR*, *IGF-I*, *IGFBP3*, *JAK2*, *STAT5b* and *STAT5a*.



Supplementary Figure S4 | GHBP levels in the F2 generation. The five-month-old -/- pigs showed an absence of GHBP in plasma. The -/-, +/- ($GHR^{+/4bp}$) and +/+ ($GHR^{+/+}$) represent different genotypes in the F2 generation; the bars represent the means \pm SD (at least three pigs per group); ** $P < 0.01$, * $P < 0.05$.



Supplementary Figure S5 | Reduced bone growth in the -/- pigs. The femur and tibia from three-and-half-month-old pigs are shown; the -/-, +/- (GHR^{+4bp}) and +/+ ($GHR^{+/+}$) represent different genotypes in the F2 generation; with one pig per group.