Supplementary Material

Growth hormone receptor-deficient pigs resemble the pathophysiology of human Laron syndrome and reveal altered activation of signaling cascades in the liver

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Supplementary Table 1. Antibodies employed for Western blot analyses.

Antigen	Antibody	Host	Dilution
pJAK2	Cell Signaling, Frankfurt, Germany #3776	rabbit	1:2000
JAK2	Cell Signaling, Frankfurt, Germany #3230	rabbit	1:2000
pMAPK	Cell Signaling, Frankfurt, Germany #4370	rabbit	1:2000
MAPK	Cell Signaling, Frankfurt, Germany #9102	rabbit	1:2000
pSTAT1	Cell Signaling, Frankfurt, Germany #7649	rabbit	1:2000
STAT1	Cell Signaling, Frankfurt, Germany #14994	rabbit	1:2000
pSTAT3	Cell Signaling, Frankfurt, Germany #9145	rabbit	1:2000
STAT3	Cell Signaling, Frankfurt, Germany #12640	rabbit	1:2000
pSTAT5	Cell Signaling, Frankfurt, Germany #9359	rabbit	1:2000
STAT5	Cell Signaling, Frankfurt, Germany #94205	rabbit	1:2000
pPI3K	Cell Signaling, Frankfurt, Germany #4228	rabbit	1:2000
PI3K	Cell Signaling, Frankfurt, Germany #4257	rabbit	1:2000
pAKT	Cell Signaling, Frankfurt, Germany #4060	rabbit	1:2000
AKT	Cell Signaling, Frankfurt, Germany #4691	rabbit	1:2000
pmTOR	Cell Signaling, Frankfurt, Germany #5536	rabbit	1:1000
mTOR	Cell Signaling, Frankfurt, Germany #2983	rabbit	1:1000
pAMPK	Cell Signaling, Frankfurt, Germany #2535	rabbit	1:2000
AMPK	Cell Signaling, Frankfurt, Germany #2532	rabbit	1:2000
pS6K	Cell Signaling, Frankfurt, Germany #9205	rabbit	1:2000
pS6K	Cell Signaling, Frankfurt, Germany #2708	rabbit	1:2000
p4EBP1	Cell Signaling, Frankfurt, Germany #2855	rabbit	1:2000
4EBP1	Cell Signaling, Frankfurt, Germany #9644	rabbit	1:2000
eIF4E	Cell Signaling, Frankfurt, Germany #9742	rabbit	1:2000
PPARG	Cell Signaling, Frankfurt, Germany #2435	rabbit	1:2000
pGSK3B	Cell Signaling, Frankfurt, Germany #9322	rabbit	1:2000
GSK3B	Cell Signaling, Frankfurt, Germany #9315	rabbit	1:2000
pINSR	Cell Signaling, Frankfurt, Germany #3024	rabbit	1:2000
INSR	Cell Signaling, Frankfurt, Germany #3025	rabbit	1:2000
pIRS1	Cell Signaling, Frankfurt, Germany #3203	rabbit	1:2000
IRS1	Cell Signaling, Frankfurt, Germany #3407	rabbit	1:2000
pLEPR	Merck, Darmstadt, Germany #07-1317	rabbit	1:500
LEPR	Bio-Rad, Munich, Germany #AHP1396	goat	1:1000

Supplementary Table 2. Factors affecting body fat content, muscle-to-back fat ratio, and serum leptin levels of 6-month-old control and *GHR*-KO pigs.

Parameter	Control			*GHR*-KO			Factors (p values)		
	Sex	mean	SD	Sex	mean	SD	Group	Sex	Group*Sex
Body fat (%)	male	9.6	1.1	male	20.9	2.2	< 0.0001	0.0108	0.1543
	female	13.2	2.5	female	22.0	4.1			
Muscle-to-back fat ratio (v/v)	male	8.6	2.3	male	2.9	0.5	< 0.0001	0.6289	0.4335
	female	7.7	2.3	female	3.1	1.3			
Serum leptin (µg/L)	male	3.0	1.0	male	4.8	2.7	0.0066	0.013	0.7437
	female	4.7	1.2	female	6.1	1.6			

The table shows means and standard deviations (SD) of means for body fat (%) and muscle to fat ratio calculated for 11 male/14 female control pigs and for 6 male/6 female *GHR*-KO pigs. Means and SD for fasting serum leptin were calculated for 9 male/13 female control and 6 male/6 female *GHR*-KO pigs. Data were analyzed using the General Linear Models Procedure (PROC GLM) as described in section 2.8 of the main manuscript.

Supplementary Table 3. Organ weights of control and *GHR*-KO pigs.

	Control (n = 25)	*GHR*-KO (n = 9)	% of Control	p value
Absolute weights (g)				
Liver	2315.5 ± 80.4	711.5 ± 133.9	30.6 ± 1.8	< 0.0001
Kidneys*	335.7 ± 11.0	102.4 ± 18.4	30.7 ± 1.4	< 0.0001
Heart	356.6 ± 9.9	133.0 ± 16.4	37.3 ± 2.1	< 0.0001
Brain	99.3 ± 1.5	83.4 ± 2.5	84.1 ± 2.0	< 0.0001
Lungs	686.2 ± 21.6	257.6 ± 34.9	37.1 ± 2.2	< 0.0001
Spleen	491.6 ± 28.9	177.6 ± 48.0	36.1 ± 4.1	< 0.0001
Pituitary gland	0.27 ± 0.01	0.13 ± 0.01	46.2 ± 1.8	< 0.0001
Adrenal glands*	5.56 ± 0.30	2.10 ± 0.50	37.0 ± 3.0	< 0.0001
Thyroid gland	7.45 ± 0.30	3.05 ± 0.60	40.4 ± 2.6	< 0.0001
Relative weights (% of body weight)				
Liver	2.53 ± 0.10	1.85 ± 0.20	72.8 ± 2.8	0.0005
Kidneys*	0.37 ± 0.01	0.27 ± 0.02	74.1 ± 3.8	0.0002
Heart	0.39 ± 0.01	0.34 ± 0.01	89.1 ± 2.1	0.0119
Brain	0.11 ± 0.005	0.22 ± 0.01	200.7 ± 10.0	< 0.0001
Lungs	0.75 ± 0.03	0.68 ± 0.05	89.3 ± 6.7	0.2304
Spleen	0.54 ± 0.03	0.46 ± 0.06	86.1 ± 9.2	0.2525
Pituitary gland	2.90 E-4 ± 0.09 E-4	3.30 E-4 ± 0.15 E-4	110.2 ± 4.0	0.0899
Adrenal glands*	6.10 E-3 ± 0.36 E-3	5.50 E-3 ± 0.65 E-3	88.7 ± 5.6	0.4267
Thyroid gland	8.20 E-3 ± 0.45 E-3	8.20 E-3 ± 0.80 E-3	98.2 ± 7.7	0.9384

Data are presented as least squares means (LSM) and standard errors (SE) of LSM estimated for the 2 groups (the statistical model used is described in section 2.8 of the main manuscript). LSMs were compared using Student's *t*-tests. *Cumulative weight of both organs.

Supplementary Table 4. Factors affecting parameters of lipid metabolism of control and *GHR*-KO pigs.

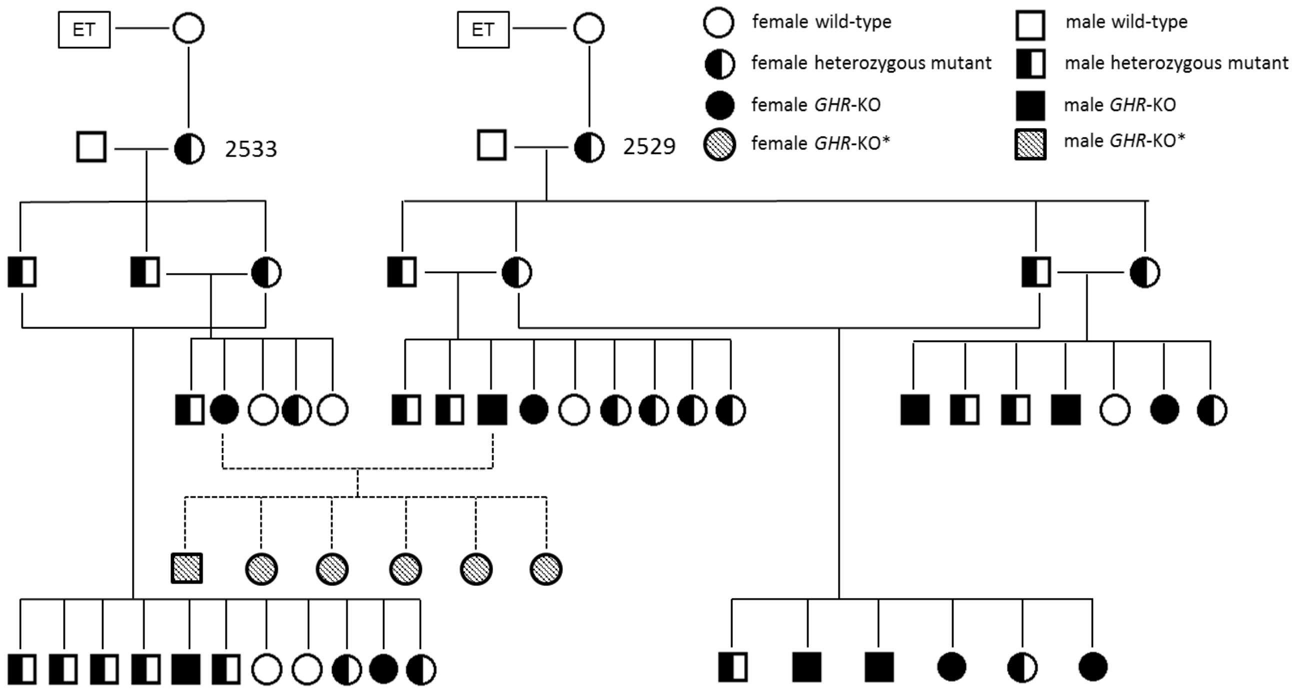
Parameter	Age	Sex	Control		*GHR*-KO		Factors (p values)				
			mean	SD	mean	SD	Group	Sex	Age	Group*Age	Group*Sex*Age
Triglycerides (mg/dL)	1	male	40.3	16.5	20.5	6.1	0.0113	0.2435	0.7666	0.301	0.9918
		female	44.3	20.5	25.8	10.9					
	2	male	34.8	14.6	26.2	8.1					
		female	42.1	20.3	33.9	11.0					
Cholesterol (mg/dL)	1	male	76.6	7.9	61.6	4.6	0.0851	< 0.0001	0.0535	0.0291	0.6255
		female	86.9	4.7	78.4	4.8					
	2	male	60.4	11.4	60.6	7.9					
		female	78.2	10.4	81.0	6.7					
LDL (mg/dL)	1	male	47.1	5.8	38.3	3.9	0.0971	< 0.0001	0.1097	0.1181	0.6526
		female	53.2	3.1	47.4	2.9					
	2	male	36.7	8.1	36.6	5.1					
		female	49.2	9.8	48.9	3.4					
HDL (mg/dL)	1	male	31.2	3.0	26.6	4.6	0.7111	0.0002	0.5071	0.0022	0.4577
		female	36.5	3.2	31.4	4.2					
	2	male	26.9	5.1	28.1	4.7					
		female	30.3	2.2	36.9	2.9					

The table shows means and standard deviations (SD) of means calculated for 6 male/4 female control pigs and 3 male/3 female *GHR*-KO pigs at Age 1 (12-15 weeks) and for 8 male/6 female control pigs and 5 male/4 female *GHR*-KO pigs at Age 2 (23-27 weeks). Data were analyzed using the General Linear Models Procedure (PROC GLM) as described in section 2.8 of the main manuscript.

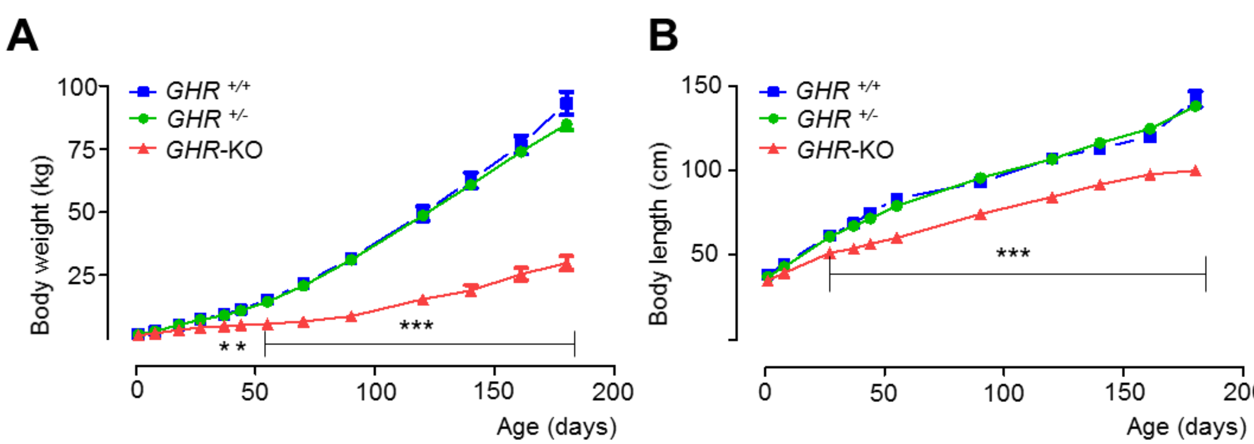
Supplementary Table 5. Clinical-chemical parameters of 6-month-old control and *GHR*-KO pigs.

Parameter [unit]	Control (n = 14)	*GHR*-KO (n = 9)	p value	Normal range
Urea [mmol/L]	4.3 ± 0.3	7.4 ± 0.5	< 0.0001	3.2 – 8.2
Creatinine [µmol/L]	126.4 ± 5.0	91.2 ± 6.5	0.0004	40 – 133
Total protein [g/L]	65.3 ± 2.2	63.6 ± 2.9	0.6381	55 – 85
Albumin [g/L]	40.1 ± 1.4	39.8 ± 1.8	0.8957	17.3 – 43.3
Bilirubin [µmol/L]	1.8 ± 0.1	1.5 ± 0.2	0.2351	< 4.3
GGT [U/L]	36.4 ± 1.8	32.5 ± 2.4	0.2131	0 – 40
Sodium [mmol/L]	141.5 ± 1.0	139.9 ± 1.3	0.3330	133 – 150
Potassium [mmol/L]	5.0 ± 0.2	4.2 ± 0.3	0.0602	4.4 – 6.7
Chloride [mmol/L]	101.8 ± 0.9	99.6 ± 1.2	0.1586	95 – 110
Calcium [mmol/L]	2.6 ± 0.04	2.7 ± 0.05	0.1744	2.4 – 3.0
Magnesium [mmol/L]	1.1 ± 0.04	1.1 ± 0.05	0.2740	0.5 – 1.2
Phosphorus [mmol/L]	2.4 ± 0.1	2.2 ± 0.04	0.1063	2.1 – 3.3
Iron [µmol/L]	17.7 ± 1.2	23.6 ± 1.6	0.0094	> 17.9

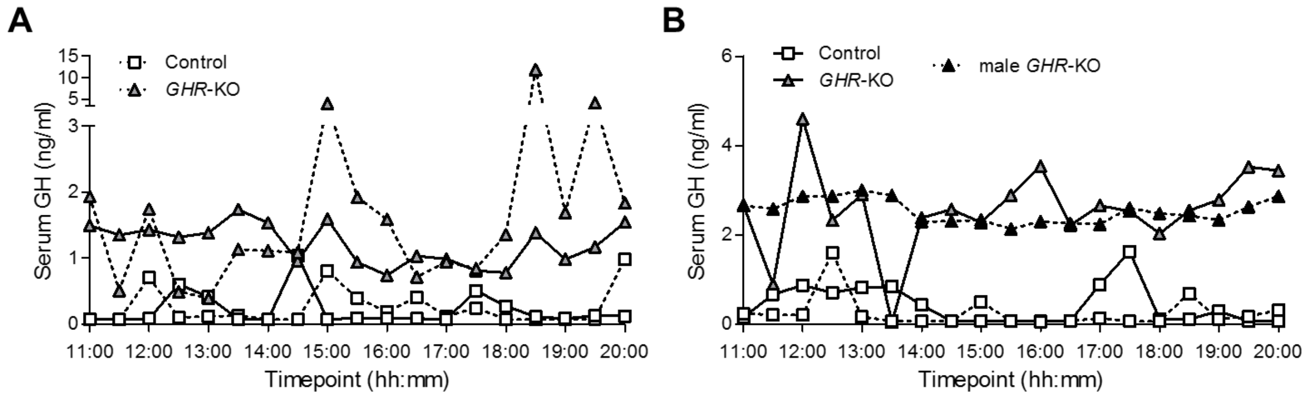
Data are presented as least squares means (LSM) and standard errors (SE) of LSM estimated for the 2 groups. The statistical model used is described in section 2.8 of the main manuscript. LSM were compared using Student's t-test.



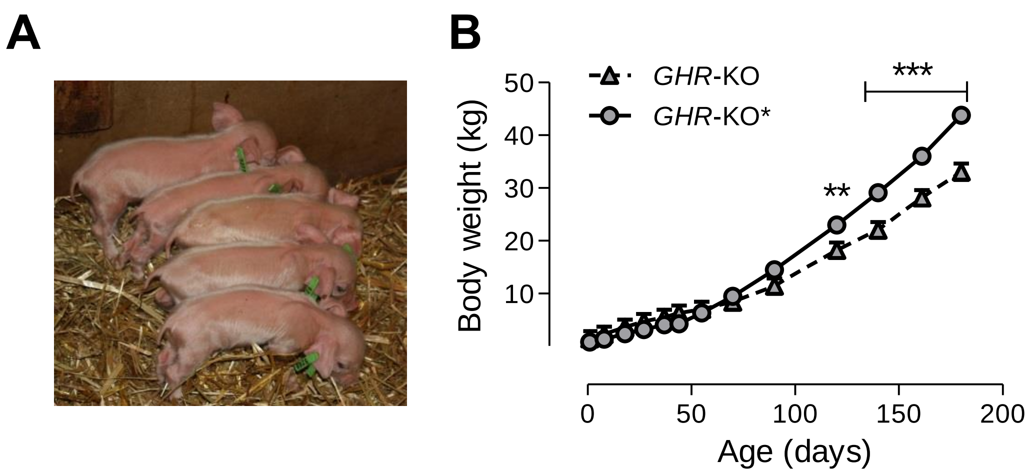
Supplementary Figure 1. Breeding scheme of *GHR*-KO animals. Heterozygous founder animals 2533 and 2539 were mated with wild-type boars and their heterozygous offspring were intercrossed to produce *GHR*-KO animals as well as wild-type and heterozygous *GHR* mutant littermates. The mating of two *GHR*-KO animals resulted in a litter of six healthy *GHR*-KO* piglets.



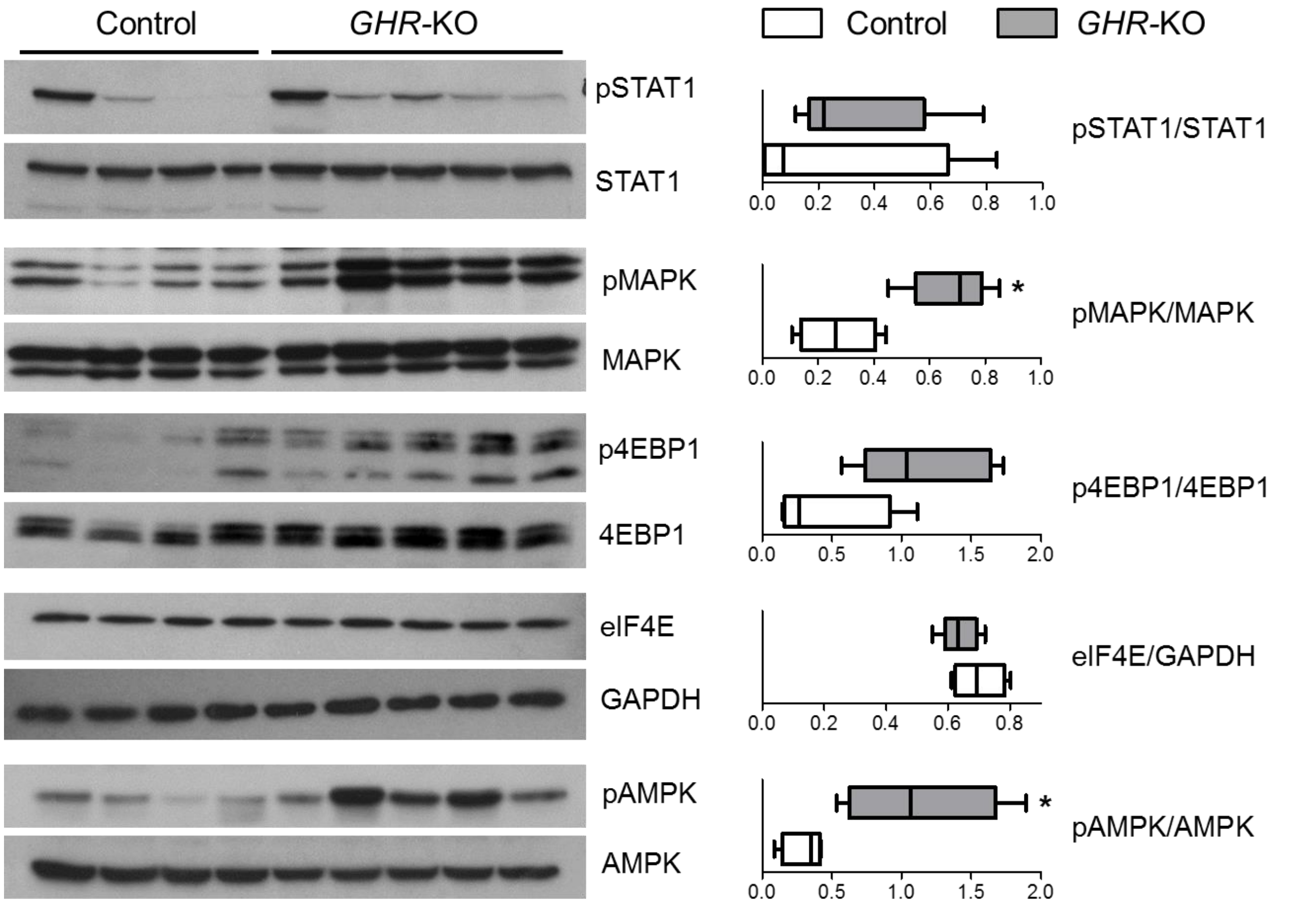
Supplementary Figure 2. Body weight gain (A) and longitudinal growth (B) of *GHR*-KO (n = 12) compared to wild-type (*GHR* $^{+/+}$; n = 6) and heterozygous *GHR* mutant (*GHR* $^{+/-}$; n = 19) pigs. Note that there was no significant difference in growth of *GHR* $^{+/+}$ and *GHR* $^{+/-}$ pigs. The two groups were thus pooled as GHR expressing control group.



Supplementary Figure 3. GH secretion profiles of additional *GHR*-KO (n=4) and control pigs (n=4). (A) Secretion pattern of two female *GHR*-KO and control pigs. (B) Secretion pattern of a female and male *GHR*-KO and two female control pigs.



Supplementary Figure 4. *GHR*-KO animals are able to reproduce. Mating of a *GHR*-KO sow and *GHR*-KO boar resulted in a litter of healthy *GHR*-KO* piglets. **(A)** Picture of *GHR*-KO* piglets at the age of one day. **(B)** *GHR*-KO* piglets had lower birth weights than *GHR*-KO piglets from heterozygote x heterozygote mating, but reached a higher weight at 6 months of age (*GHR*-KO: n = 12, *GHR*-KO*: n = 6). Data are presented as least squares means (LSM) and standard errors (SE) of LSM estimated for the 2 groups. The statistical model used is described in section 2.8 of the main manuscript. LSMs were compared using Student’s t-test.



Supplementary Figure 5. Western blot analysis of GHR-related signaling molecules in liver samples of 6-month-old fasted *GHR*-KO (n=5) and control pigs (n=4). The box plots show medians, 25th and 75th percentiles (box), and extremes (whiskers). * $p < 0.05$; evaluated using Mann-Whitney U test.