SUPPLEMENTARY INFORMATION

Blocking FGF23 signaling improves the growth plate of mice with X-linked hypophosphatemia

SUPPLEMENTARY FIGURES

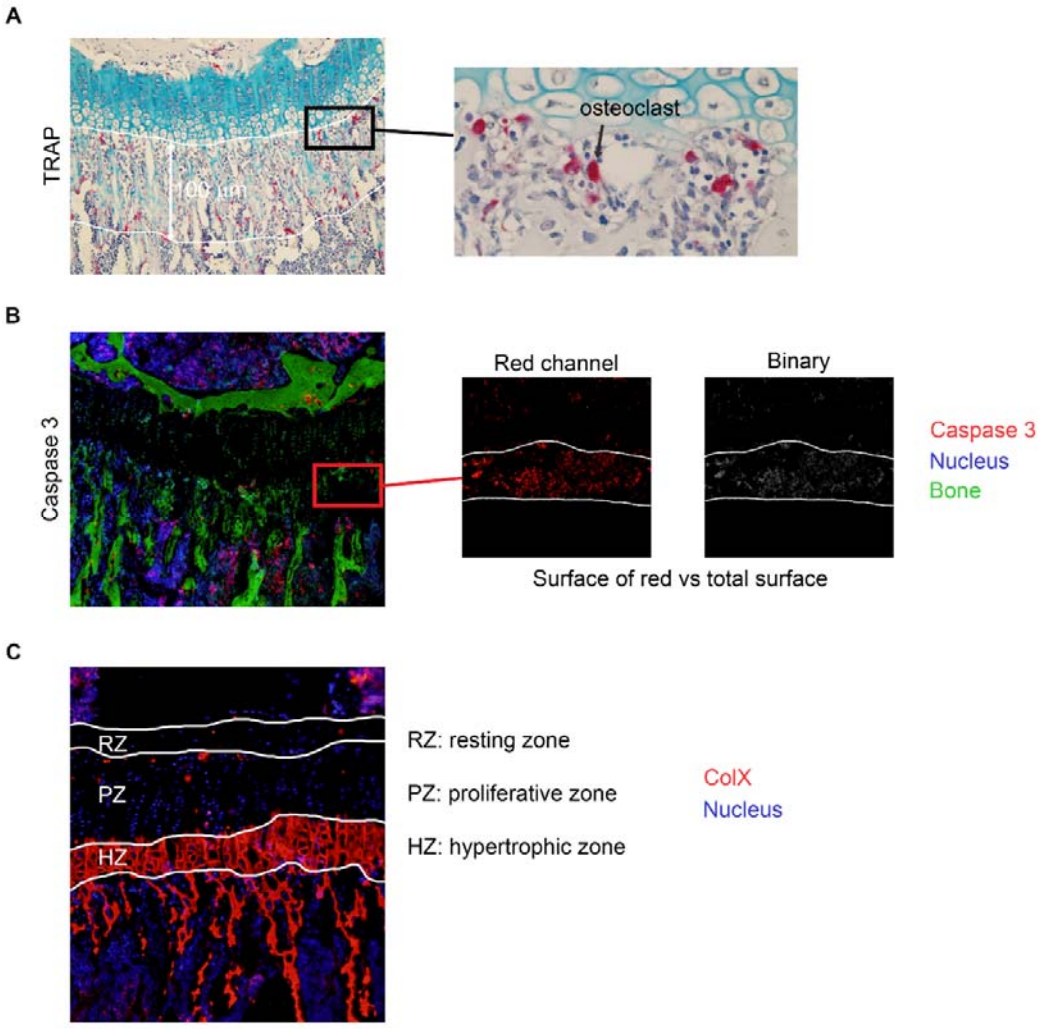

Figure S1. TRAP staining and immunofluorescence determinations. **(**A) Quantification of the red (TRAP) stained area which indicates active osteoclasts, TRAP positive cells, as the fraction of the total area comprising 100 µm of the growth plate. The growth plate is delineated with white lines. (B) Caspase 3 immunofluorescence quantification: in the hypertrophic zone several areas of equal size were selected, the red channel was converted to greyscale channel using the Fiji Software[1] and the threshold area versus the total area was determined as the Caspase 3 surface fraction (%). (C) Collagen X (ColX) immunofluorescence quantification: determination of the height of the collagen X-stained region in red which represents the hypertrophic zone in the growth plate (marked in white and labeled as HZ). The average quantification for each mouse was calculated from around 20 determinations.

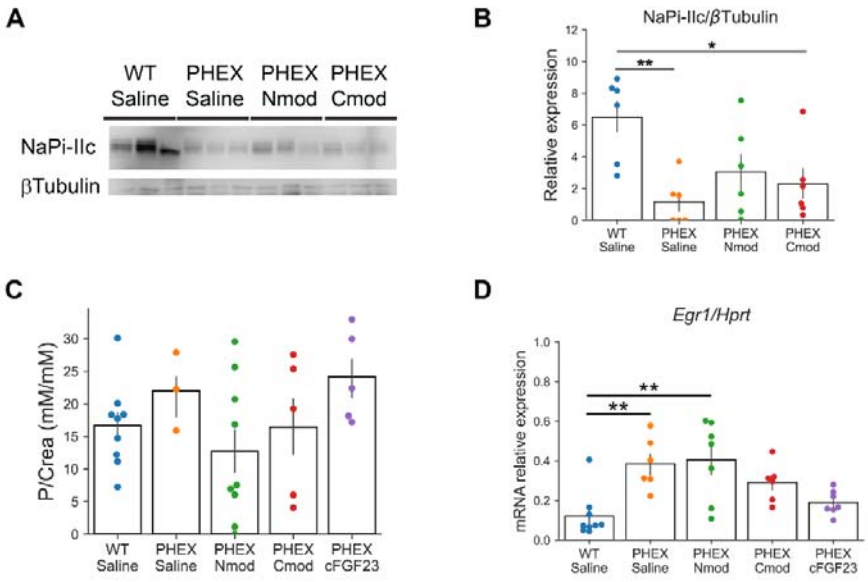

Figure S2. (A-B) Representative Western blot images of NaPi-IIc and β-tubulin protein expression in brush border membranes of kidneys from WT and PHEX mice after 7 days treatment and densitometry analysis (n = 6 mice/group). (C) Phosphate urinary excretion normalized to creatinine excretion in urine in WT and PHEX mice after 7 days treatment (n = 3-9 mice/group). (D) *Egr1* mRNA expression in kidneys from WT and PHEX mice after 7 days treatment (n = 6-9 mice/group). cFGF23: C-terminal FGF23 fragment residues S180 to S250, Nmod: N-acetylation of cFGF23 peptide, Cmod: C-amidation of cFGF23 peptide. Tukey HSD P value: * < 0.5, ** < 0.01.

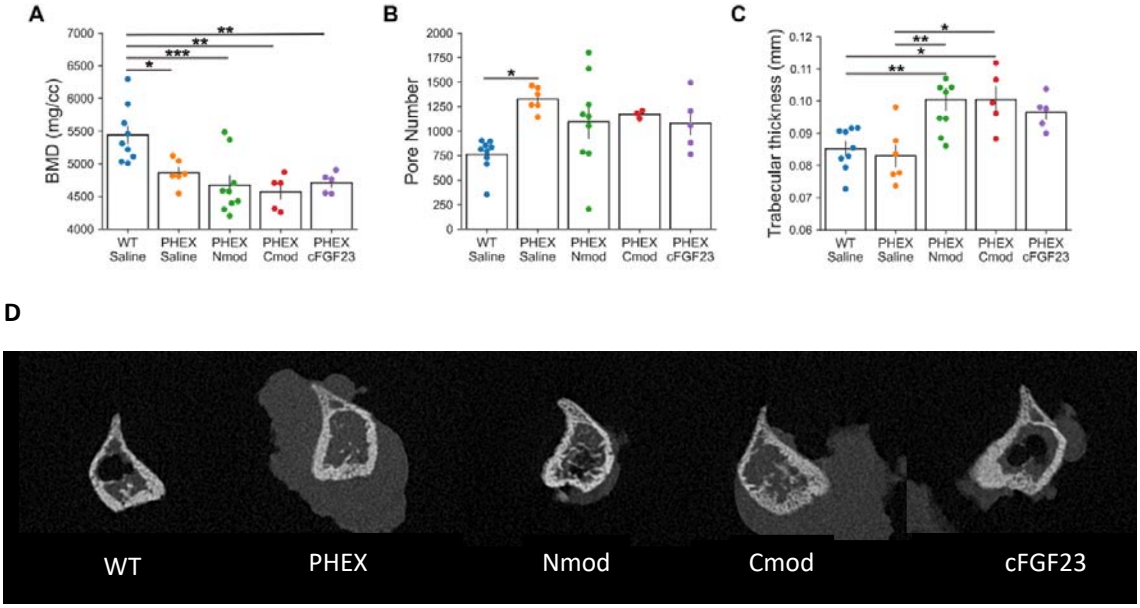

Figure S3. Treated PHEX mice showed no differences in bone mineral density. (A-C) Bone mineral density, pore number, and trabecular thickness of tibias assessed by microcomputer tomography from WT and PHEX mice after 7 days treatment with saline solution or 1mg/kg FGF23 fragments (n = 5-9 mice/group). (D) Representative images of microcomputer tomography analysis. cFGF23: C-terminal FGF23 fragment residues S180 to S250, Nmod: N-acetylation of cFGF23 peptide, Cmod: C-amidation of cFGF23 peptide. Tukey HSD P value: * < 0.5, ** < 0.01, *** < 0.001.

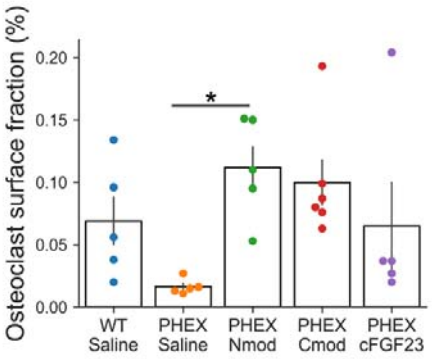

Figure S4. *Phex*^{C733RMhda} (PHEX) mice treated with C-terminal FGF23 fragments increased their osteoclast activity. Osteoclast surface fraction after 7 days treatment with saline solution or 1mg/kg FGF23 fragments (n = 4-5 mice/group). cFGF23: C-terminal FGF23 fragment residues S180 to S250, Nmod: N-acetylation of cFGF23 peptide, Cmod: C-amidation of cFGF23 peptide. Tukey HSD P value: * < 0.5.

SUPPLEMENTARY TABLES

TABLE S1. Commercial Kits

Name	Company	Catalog Number
Mouse/Rat FGF-23 (Intact) Kit	Immutopics International	60-6800
Mouse intact PTH ELISA kit	Immutopics International	60-2305
1,25-Dihydroxy Vitamin D RIA kit	Immunodiagnostic System	AA-54F1
Urea Assay Kit	Sigma-Aldrich	MAK006-1KT
QuantiChrom Assay Kit	Bio-Assays	DiHM-250

TABLE S2. Primers

Gene	Accession Number	Forward Primer	Reverse Primer
EGR1	NM_001964.3	5‘-AGC AGC ACC TTC AAC CCT C-3‘	5‘-GTC TCC ACC AGC ACC TTC TC-3‘
HPRT1	NM_000194.3	5'- AAG GGT GTT TAT TCC TCA TGG ACT A-3'	5'- GGC CTC CCA TCT CCT TCA TC-3'

TABLE S3. Antibodies

Name	Company	Catalog Number	Dilution	Use
BrdU	ThermoFischer	B3518	1:200	IF
Caspase 3	Cell Signaling	9661S	1:100	IF
Collagen X	Abcam	Ab58632	1:200	IF
α-Klotho	Transgenic Inc.	KO603, Clone KM2076	1:1000	WB
NaPi-IIa	Laboratory H. Murer, University of Zurich (CH)[2]	N/A	1:1000	WB
NaPi-IIc	Laboratory C.A. Wagner, University of Zurich[3]	N/A	1:1000	WB
βTubulin	Sigma-Aldrich	T7941	1:1000	WB
Anti-Rabbit secondary Ab	Perkin Elmer	NEF812001EA	1:25000	IF
Anti-Mouse IgG (H+L), HRP Conjugate	Promega	W402B	1:5000	WB
Anti-Rabbit IgG (H+L), HRP Conjugate	Promega	W401B	1:5000	WB

References

1 Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**, 676-682, doi:10.1038/nmeth.2019 (2012).

2 Custer, M., Lotscher, M., Biber, J., Murer, H. & Kaissling, B. Expression of Na-P(i) cotransport in rat kidney: localization by RT-PCR and immunohistochemistry. *Am J Physiol* **266**, F767-774, doi:10.1152/ajprenal.1994.266.5.F767 (1994).

3 Nowik, M. *et al.* Renal phosphaturia during metabolic acidosis revisited: molecular mechanisms for decreased renal phosphate reabsorption. *Pflugers Arch* **457**, 539-549, doi:10.1007/s00424-008-0530-5 (2008).