

## **Supplementary Information**

**A G protein-coupled, IP3/Protein Kinase C pathway controlling the synthesis of phosphaturic hormone FGF23**

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**Supplementary Table S1.** Sequences of primer pairs used in qRT-PCR.

Gene	Forward	Reverse
$\beta$ -Actin	5'-GATCTGGCACCACACCTTCT-3'	5'-GGGGTGTGAAGGTCTCAAA-3'
Cyp27b1	5'-CAAATGGCTTTGTCCAGAT-3'	5'-GGCTGTCTTCCGAATGGTTA-3'
Cyp24a1	5'-GTGCGGATTTCTTTGTGAT-3'	5'-GGGATTCCGGGATAGATTGT-3'
IL6	5'-TACCACTTCACAAGTCGGAGGC-3'	5'-CTGCAAGTGCATCATCGTTGTTTC-3'
XL $\alpha$ s	5'-CTCATCGACAAGCAACTGGA-3'	5'-CCCTCTCCGTAAACCCATT-3'
FGFR1	5'-TGTTTGACCGGATCTACACACA-3'	5'-CTCCCACAAGAGCACTCCAA-3'
Phex	5'-GCATGATTAACCAGTATAGCAA-3'	5'-GGTCTATAGGAATTGCACCTTAC-3'
Dmp1	5'-CGCATCCCAATATGAAGACTG-3'	5'-GCTTGACTTTCTTCTGATGACTCA-3'
Gq $\alpha$	5'-CCTTCCTATCTGCCTACACAAC-3'	5'-CCCTACATCGACCATTTCTGAAA-3'
G11 $\alpha$	5'-ATCAAGACGCTGTGGAGTG-3'	5'-TCCACGTCCGTCAAGTAGTA-3'

**Supplementary Figure Legends**

**Supplementary Figure S1.** Knockout of XL $\alpha$ s results in moderately reduced FGF23 levels at adult mice. C-terminal FGF23 levels at (A) 4-week-old, and (B) 2-month-old in WT and XLKO mice. (C) mRNA expression of Phex and Dmp1 in P10 XLKO and WT femurs (n=6 per group). \*, p<0.05; \*\*, p<0.01, calculated by unpaired two-tailed Student's t-test.

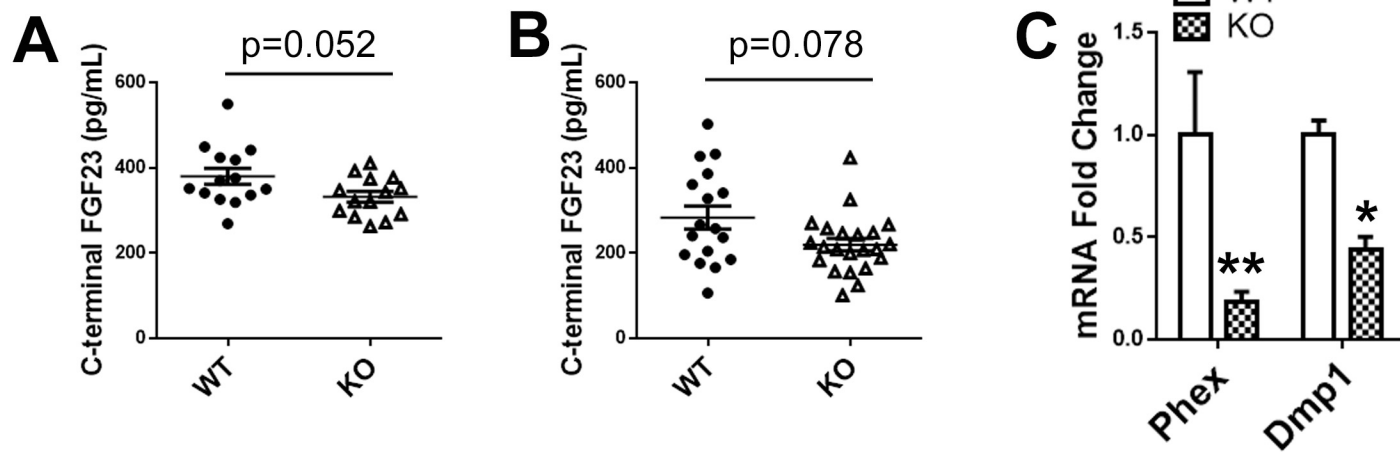
**Supplementary Figure S2.** Ablation of XL $\alpha$ s repressed IP<sub>1</sub> levels without diminishing cAMP levels. (A) cAMP levels in XL $\alpha$ s-deficient Ocy454 cells (KO clones) and control clonal cells stimulated with three different concentrations of PTH(1-34) or vehicle. PTH-induced cAMP levels were normalized to those induced by 10  $\mu$ M Forskolin in XL $\alpha$ s-deficient Ocy454 cells and control clonal cells, respectively. (B) IP<sub>1</sub> concentrations were significantly diminished in XLKO clonal Ocy454 cells at baseline. \*, p<0.05; \*\*, p<0.01, vs. control clones, significance was defined by Student's t test.

**Supplementary Figure S3.** Constitutively active Gq/PKC signaling induces FGF23 production in adult mice. (A) Serum C-terminal FGF23, and (B) skeletal FGF23 mRNA (n=8 per group) in 2-month-old GNAQ<sup>Q209L</sup>/Dmp1-Cre mice and control littermates. \*, p<0.05 vs. control littermates. (C) Relative mRNA expression of XL $\alpha$ s, Gq $\alpha$ , and G11 $\alpha$  in P10 and 8-week-old femurs (n=5~6 per group). \*, p<0.05; \*\*\*, p<0.001, calculated by unpaired two-tailed Student's t-test.

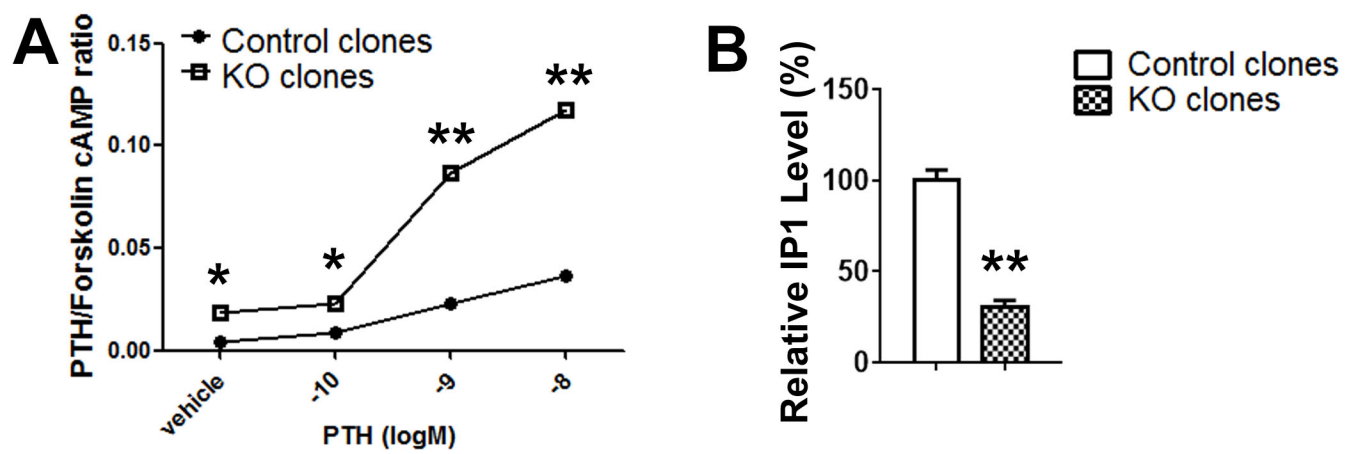
**Supplementary Figure S4.** (A) Adenovirus-mediated XL $\alpha$ s overexpression in Ocy454 cells. XL $\alpha$ s mRNA expression in control and XLKO Ocy454 cells transduced with Ad-YFP or Ad-XL $\alpha$ s. (B) SaOS-2 cells were treated with PMA, together with Bisindol, and FGF23 mRNA expression was analyzed by qRT-PCR. Statistical differences were assessed with one-way ANOVA (Tukey's).

**Supplementary Figure S5.** FGFR1 mRNA expression in XLKO femurs and XLKO Ocy454 cells are reduced, while no change is observed in FGFR1 mRNA expression in femurs of GNAQ<sup>Q209L</sup>/Ocn-Cre and GNAQ<sup>Q209L</sup>/Dmp1-Cre mice. (A, B) FGFR1 mRNA levels in femurs from (A) P10 XLKO (KO) and WT mice, and (B) in XLKO and control Ocy454 cells. \*\*, p<0.01. (C, D) Control and XLKO Ocy454 cell were stably transduced with control or FGFR1 activator sgRNA. mRNA levels of (C) FGFR1 and (D) FGF23 were assessed by qRT-PCR in these cells. Data represent means  $\pm$  SEM of values obtained from control and XLKO clones. a, p<0.05 vs. control cells with control sgRNA; b, p<0.05 vs. XLKO cells with control sgRNA. (E, F) FGFR1 mRNA expression in P10 (E) GNAQ<sup>Q209L</sup>/Ocn-Cre and littermates, and (F) GNAQ<sup>Q209L</sup>/Dmp1-Cre and control mice (n=8 per group). Statistical differences were assessed with Student's t test (A, B, E, and F), or one-way ANOVA (Tukey's) (C, D).

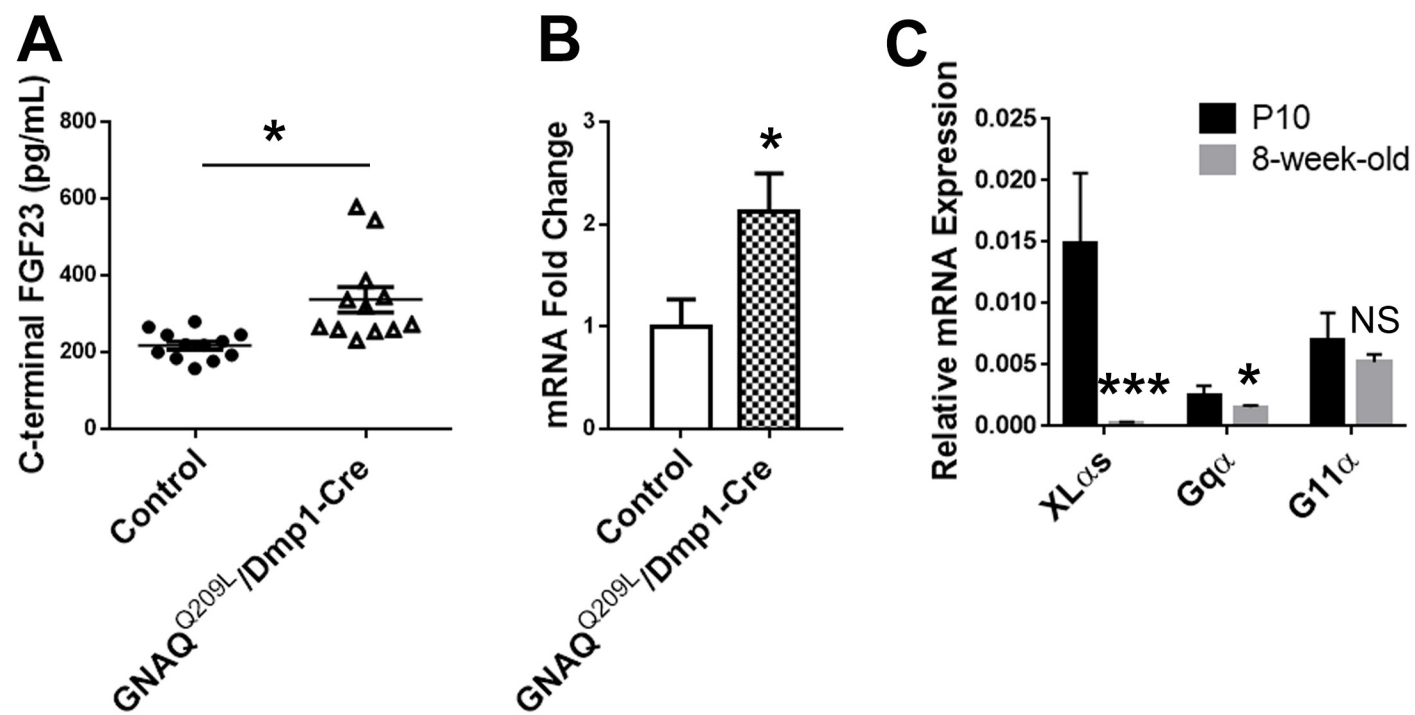
**Supplementary Figure S6.** Effects of short-term PKC inhibitor Ro32-0432 treatment in Hyp mice. (A) C-terminal FGF23 levels and (B) phosphate levels in WT and Hyp mice injected with vehicle or Ro32-0432 for twelve hours. Statistical analysis was calculated by Welch's t-test (two-tailed) followed by Bonferroni correction.



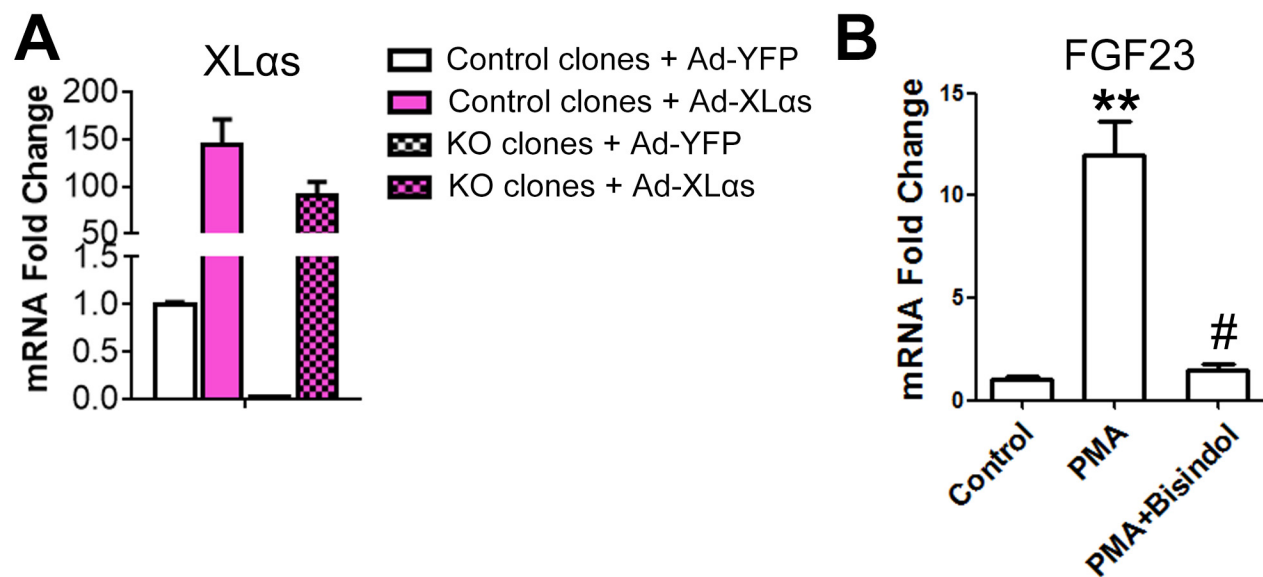
Supplementary Figure S1



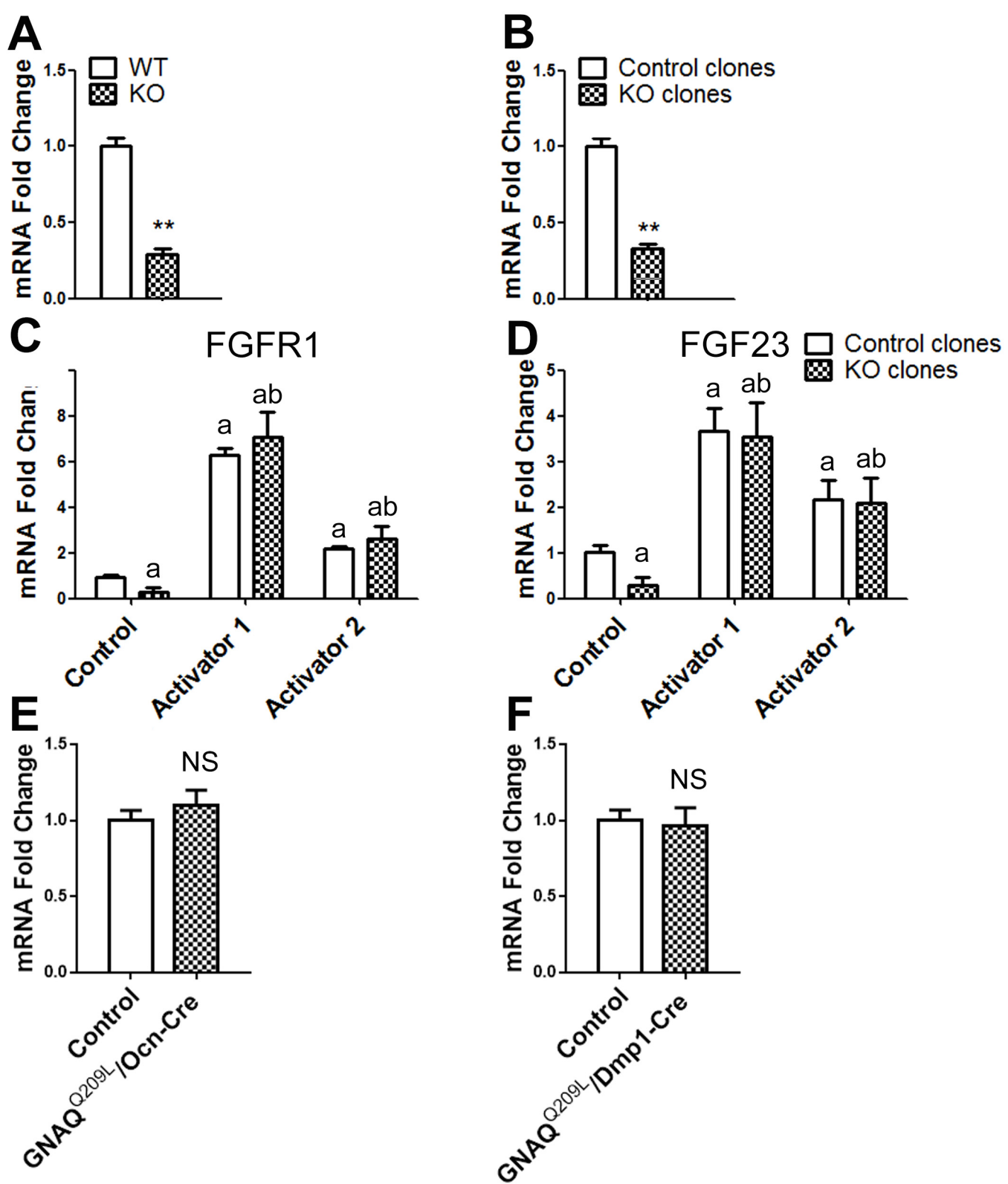
Supplementary Figure S2



Supplementary Figure S3

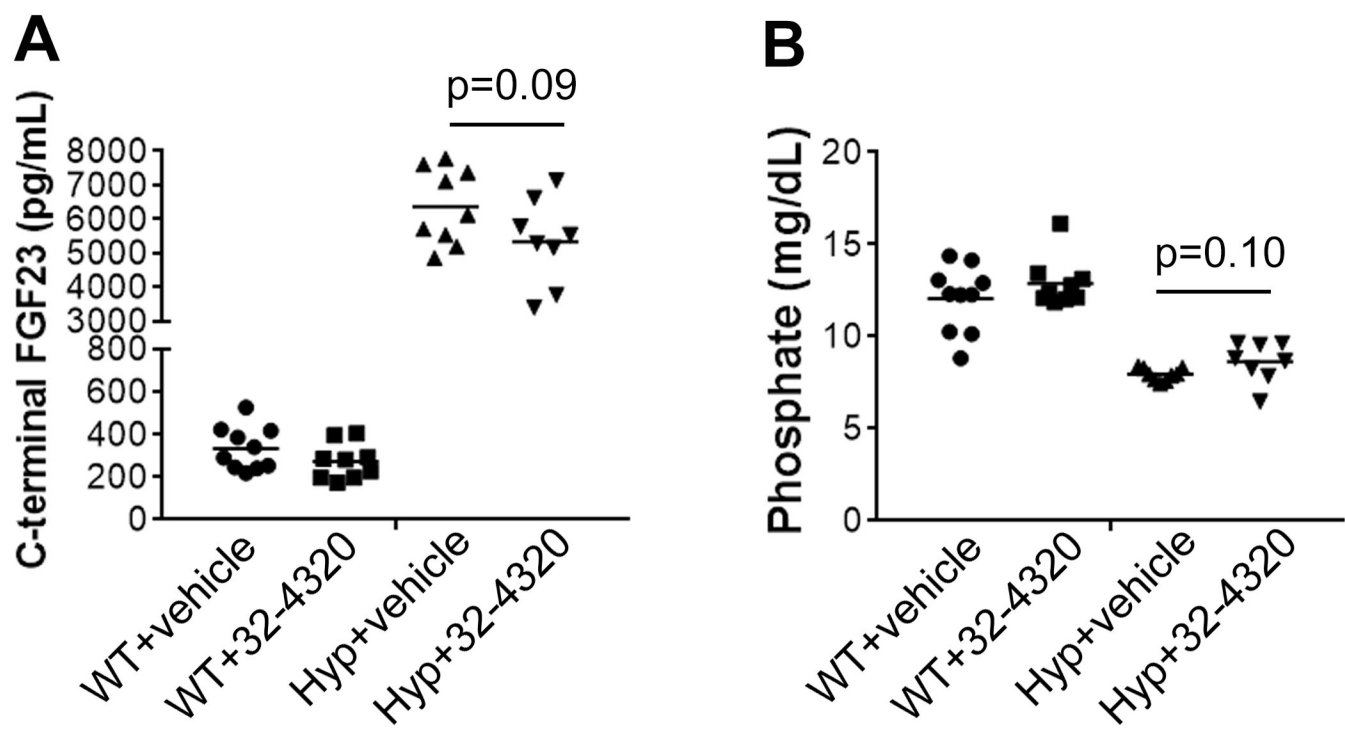


Supplementary Figure S4



Supplementary Figure S5





Supplementary Figure S6