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Supplementary Information for

Activation of unliganded FGF receptor by extracellular phosphate potentiates proteolytic protection of FGF23 by its O-glycosylation

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Figs. S1 to S10
Captions for databases S1 and S2

Other supplementary materials for this manuscript include the following:

Datasets S1 and S2

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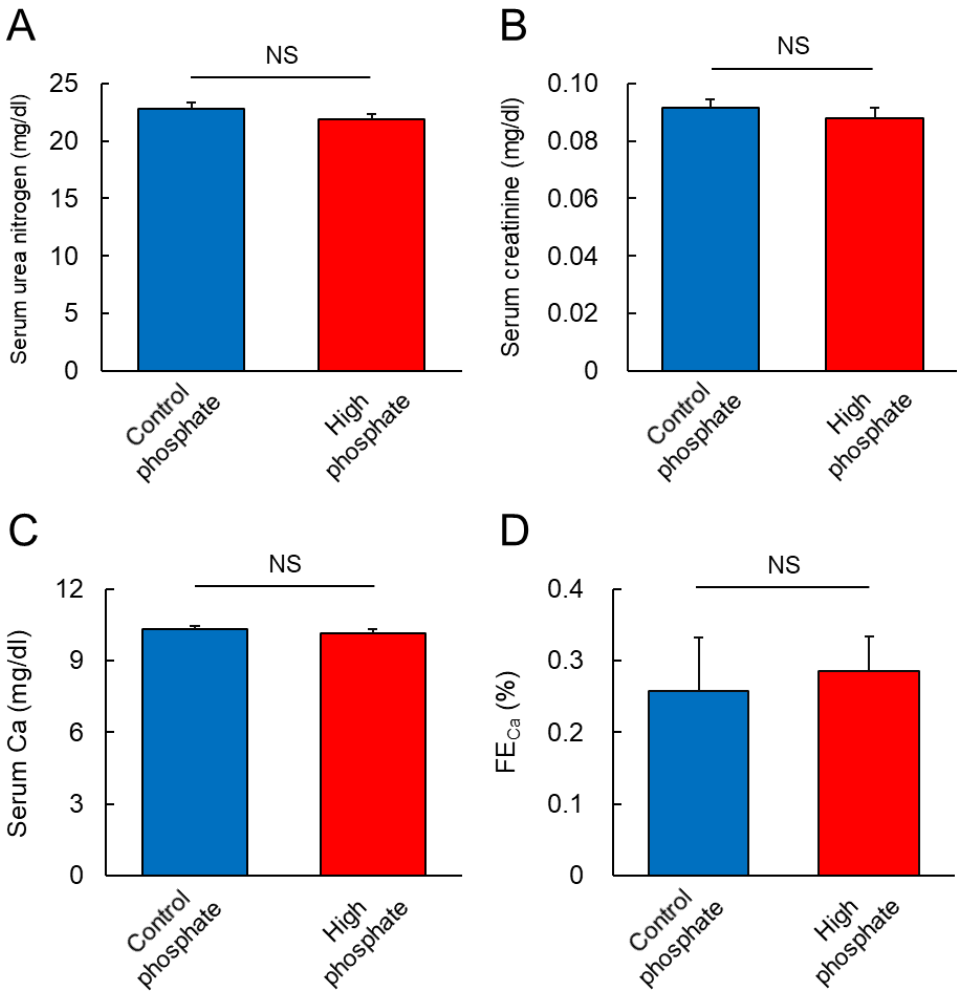

Fig. S1. A high Pi diet does not affect serum urea nitrogen, creatinine, Ca and ~~FE~~Ca in mice. (*A*) Serum urea nitrogen, (*B*) creatinine, (*C*) Ca level and (*D*) FE_{Ca} in mice fed a CP or HP diet for two weeks. Data represent the mean±SEM; *n*=19 mice per group.

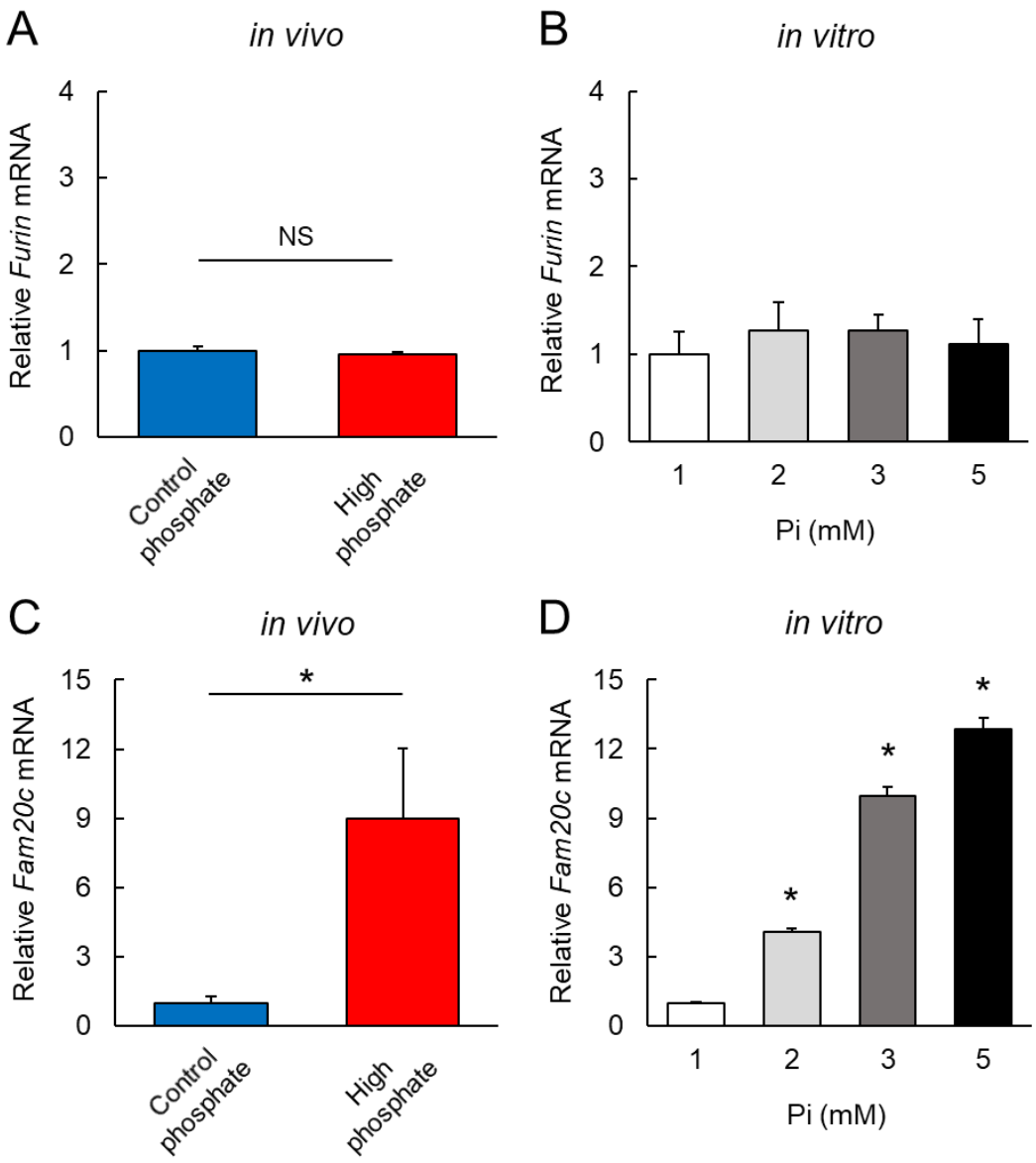

Fig. S2. High Pi does not enhance *Furin* but increase *Fam20c* expression *in vivo* and *in vitro*. (*A* and *C*) *Furin* (*A*) and *Fam20c* (*B*) mRNA expression in femurs of mice fed with a CP or HP diet for two weeks. (*B* and *D*) *Furin* (*B*) and *Fam20c* (*D*) mRNA expression under various extracellular Pi concentrations for 48 hours in UMR106 cells. Data represent the mean±SEM. (*A* and *C*) n=19 mice per group; *P<0.05 by Student's t-test; NS: not significant. (*B* and *D*) n=3 per group; *P<0.05 by ANOVA with a *post hoc* Dunnett test compared to 1 mM of extracellular Pi.

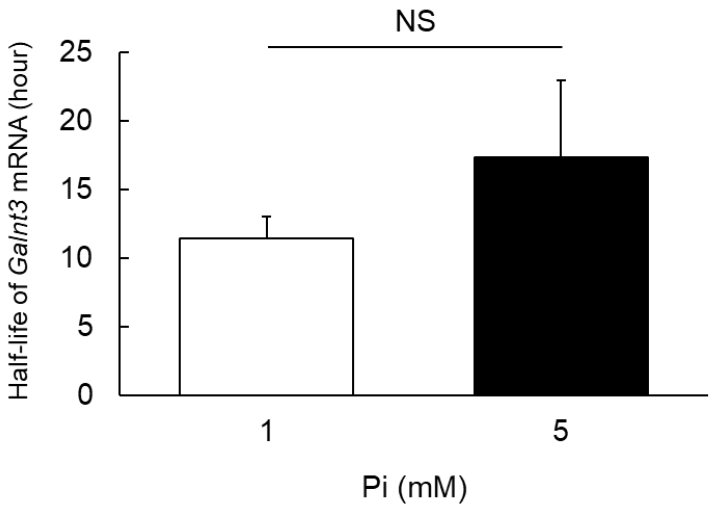

Fig. S3. High Pi does not change the half-life of *Galnt3* mRNA in UMR106 cells. The half-life was assessed by the levels of *Galnt3* mRNA for 4 hours in the presence of actinomycin D. Data represent the mean±SEM; *n*=3 per group; NS: not significant by Student's *t*-test.

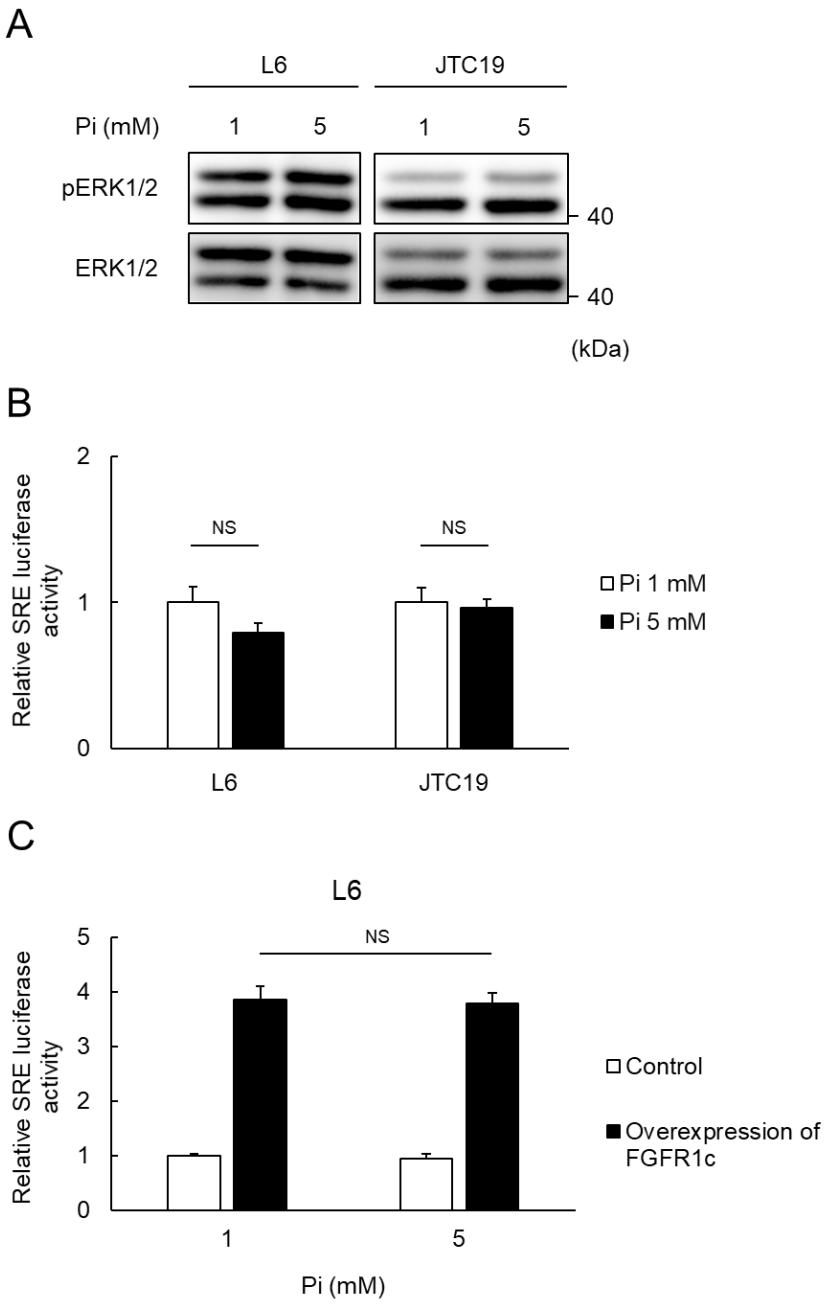

Fig. S4. No activation of ERK by high Pi in the non-osteoblastic cell lines. (*A*) Immunoblotting with an antibody to phosphorylated ERK1/2 from the lysates of L6 or JTC19 cells treated with 1 or 5 mM Pi. (*B*) The fold change of ERK activation in L6 or JTC19 cells under 1 or 5 mM Pi for 24 hours evaluated by SRE luciferase assays. (*C*) The fold change of ERK activation in L6 cells overexpressing *Fgfr1c* under 1 or 5 mM Pi for 24 hours evaluated by SRE luciferase assays. Data represent the mean±SEM. (*A*) Data are presented as a representative image. (*B* and *C*) *n*=3 per group; NS: not significant by Student's *t*-test (*B*) or by ANOVA with a *post hoc* Tukey's test (*C*).

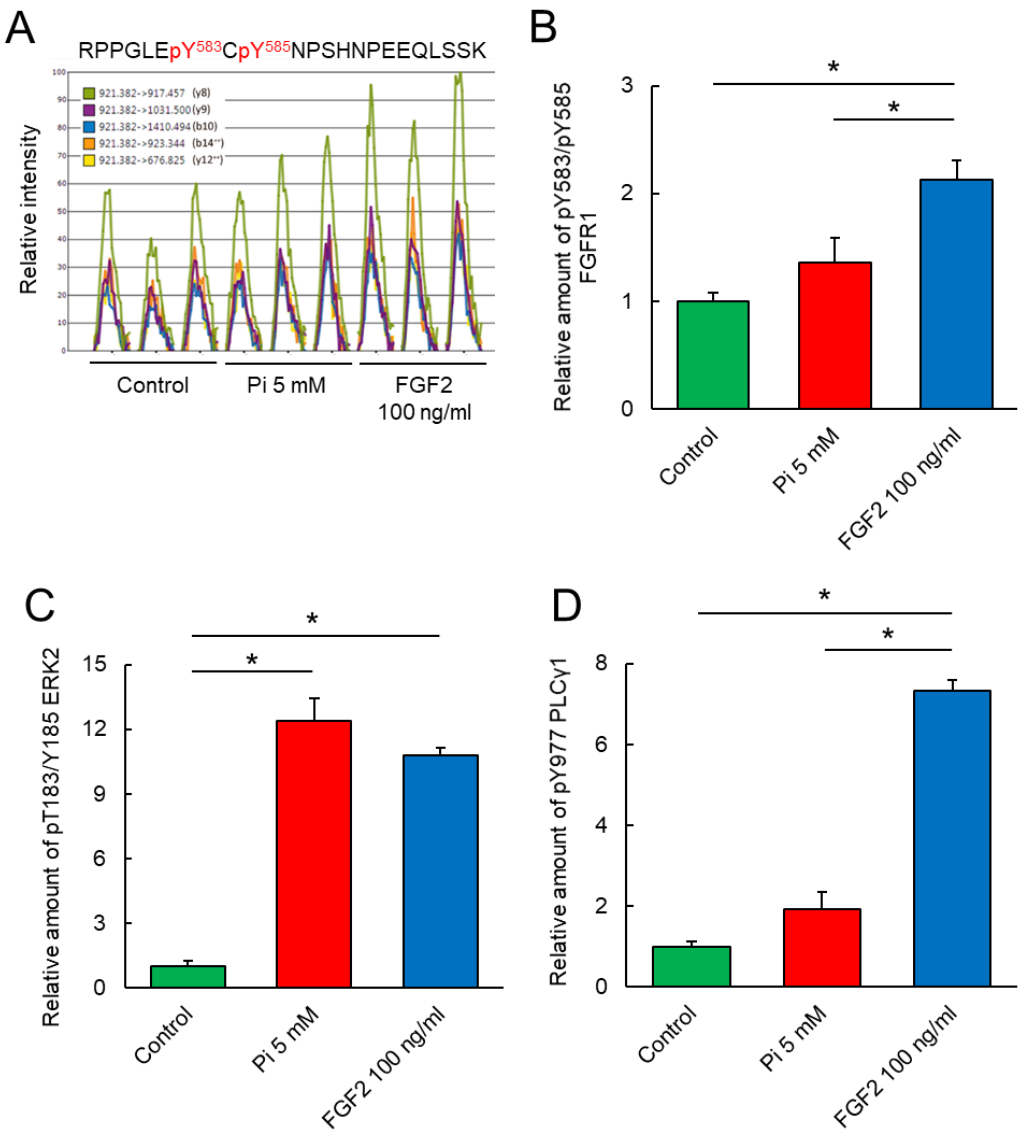

Fig. S5. The results of targeted MS using the PRM method. (*A*) Extracted ion chromatograms of transitions of the FGFR1 peptide with phosphorylated tyrosines 583 and 585. Each peptide in three independently prepared samples was analyzed by targeted MS using the PRM method. (*B-D*) The amount of peptides of FGFR1 with phosphorylated tyrosines 583 and 585 (*B*), ERK2 with phosphorylated threonine 183 and tyrosine 185 (*C*), and PLCγ with phosphorylated tyrosine 977 (*D*) normalized to the amount of peptide of cyclin-dependent kinase 2 (CDK2) with phosphorylated tyrosine 15 in the same sample. Data represent the mean±SEM. (*B-D*) n=3 per group; *P<0.05 by ANOVA with a *post hoc* Tukey's test.

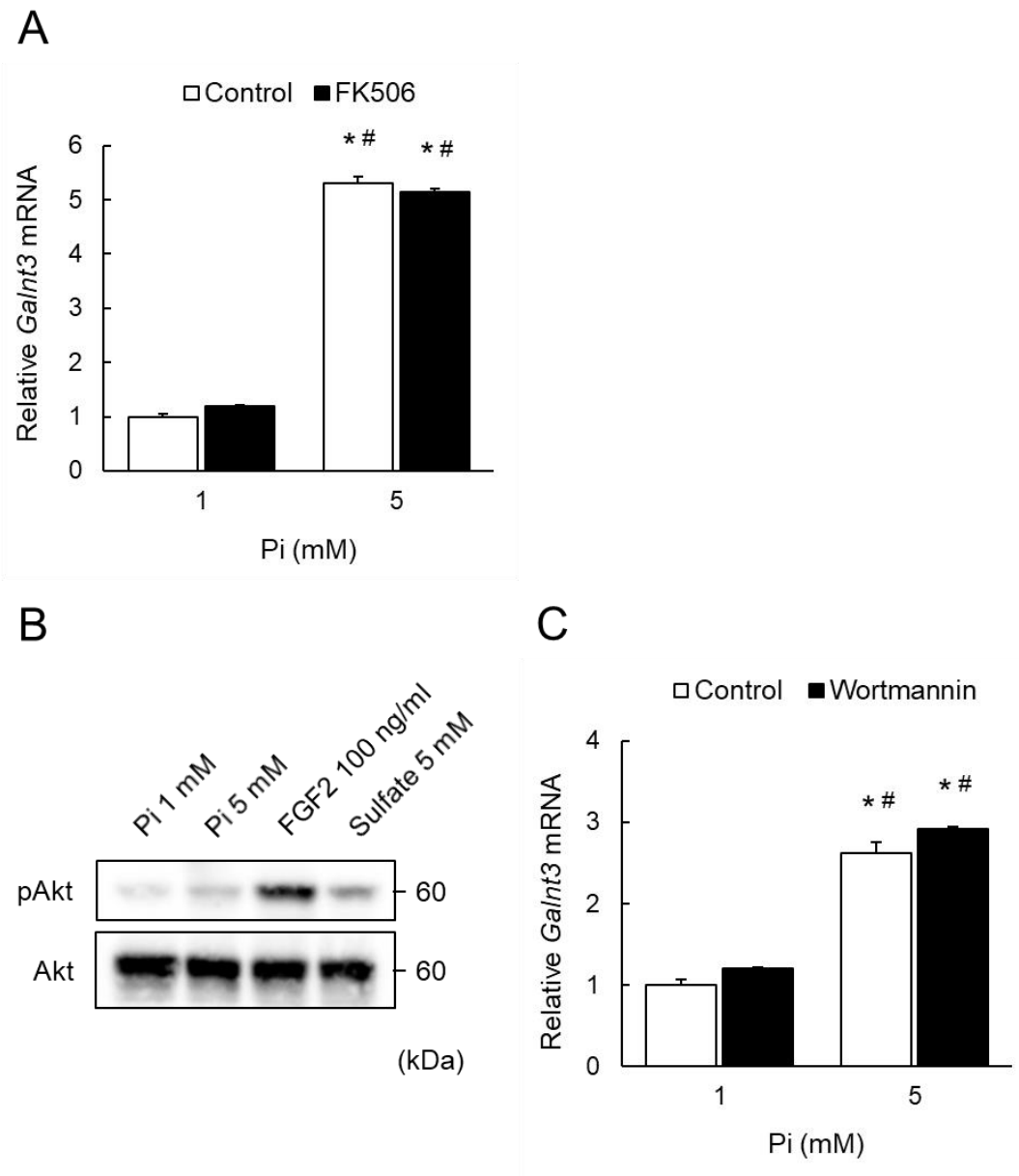

Fig. S6. The PI3K-Akt and PLCγ-calcineurin pathways are not involved in Pi sensing. (*A*) *Galnt3* mRNA expression with 5 mM Pi for 48 hours with or without the calcineurin inhibitor FK506. (*B*) Immunoblotting with an antibody to phosphorylated Akt from the lysates of UMR106 cells treated with 1 or 5 mM Pi, 100 ng/ml FGF2 or 5 mM sulfate for 15 minutes. (*C*) *Galnt3* mRNA expression with 5 mM Pi for 48 hours with or without the PI3K inhibitor wortmannin. Data represent the mean±SEM. (*A* and *C*) n=3 per group; *P<0.05 compared to 1 mM Pi without wortmannin or FK506, $^{\#}P$<0.05 compared to 1 mM Pi with wortmannin or FK506 by ANOVA with a *post hoc* Tukey's test. (*B*) Data are presented as a representative image.

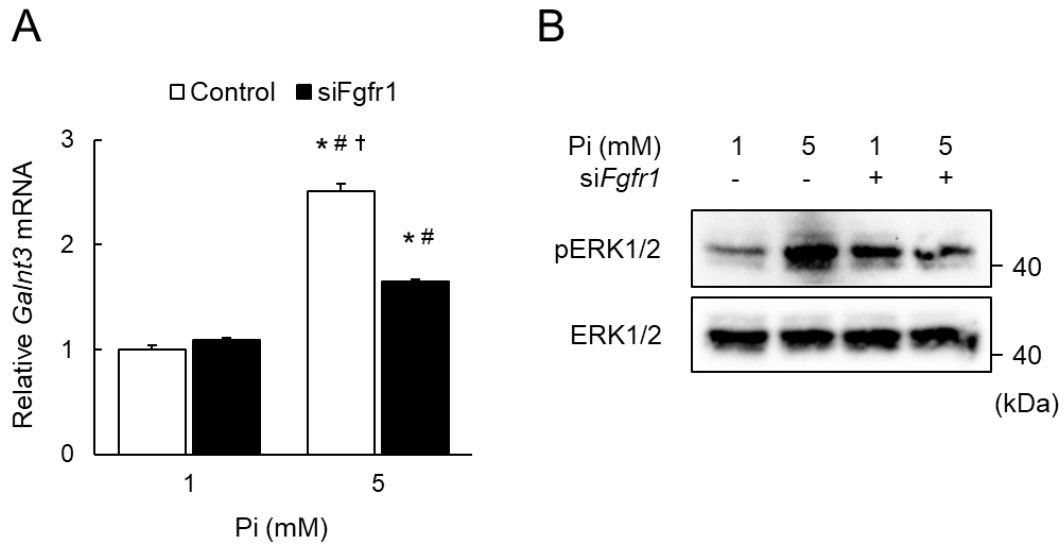

Fig. S7. Silencing *Fgfr1* by siRNA suppresses enhanced *Galnt3* expression and ERK phosphorylation by high Pi. (*A*) *Galnt3* mRNA expression by 5 mM Pi for 12 hours compared with 1 mM Pi under siRNA-mediated silencing of *Fgfr1*. (*B*) Immunoblotting with an antibody to phosphorylated ERK1/2 from the lysates of UMR106 cells under 1 or 5 mM Pi for 15 minutes with or without silencing *Fgfr1*. Data represent the mean±SEM. (*A*) n=3 per group; *P<0.05 compared to 1 mM Pi without si*Fgfr1*, $^{\#}P$<0.05 compared to 1 mM Pi with si*Fgfr1*, $^{\dagger}P$<0.05 compared to 5 mM Pi with si*Fgfr1* by ANOVA with a *post hoc* Tukey's test. (*B*) Data are presented as a representative image.

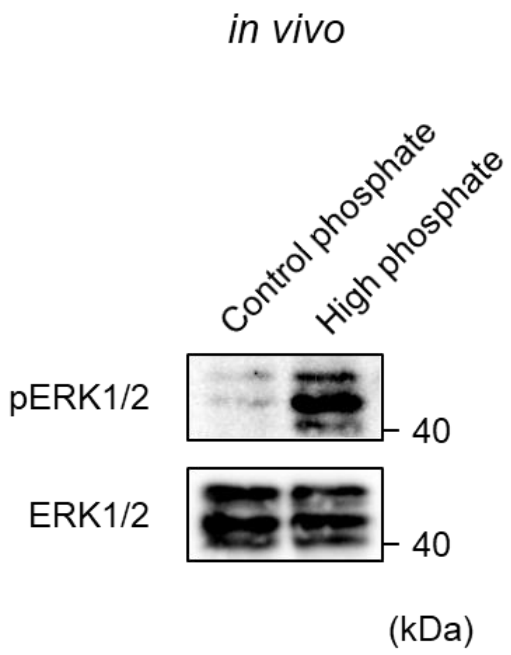

Fig. S8. A high Pi diet induces ERK phosphorylation in bone. Immunoblotting with an antibody to phosphorylated ERK1/2 from the extracts of mice femurs fed with a CP or HP for two weeks. Data are presented as a representative image.

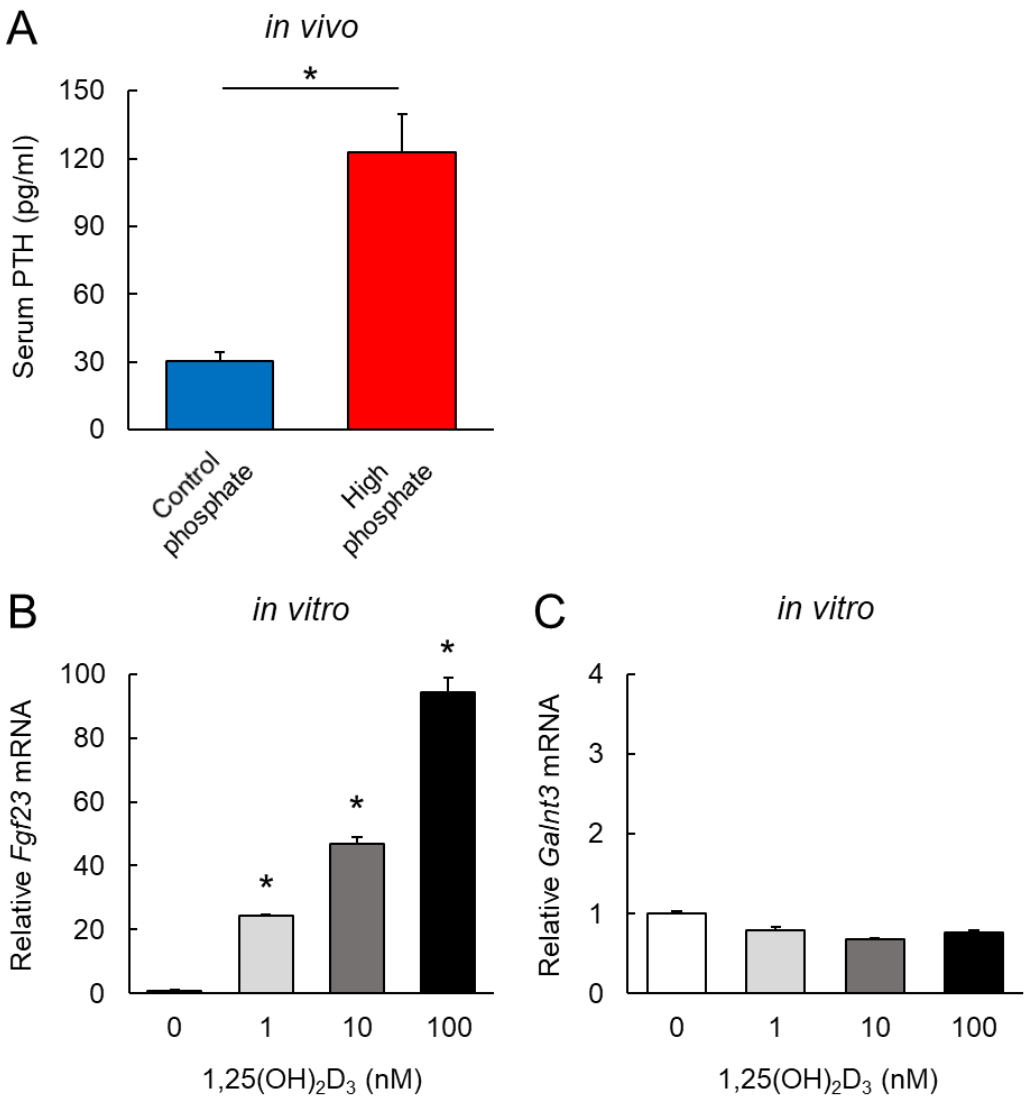

Fig. S9. Changes of other calciotropic hormones are unlikely to support the increase of *Galnt3* expression by high Pi. (*A*) Serum intact PTH (1-84) concentrations in mice fed a CP or HP diet for two weeks. (*B* and *C*) *Fgf23* (*B*) and *Galnt3* (*C*) mRNA expression under various extracellular $1,25(OH)_2D_3$ concentrations for 48 hours in UMR106 cells. Data represent the mean±SEM. (*A*) n=19 mice per group; *P<0.05 by Student's t-test. (*B* and *C*) n=3 per group; *P<0.05 by ANOVA with a *post hoc* Dunnett test compared to 0 nM of extracellular $1,25(OH)_2D_3$.

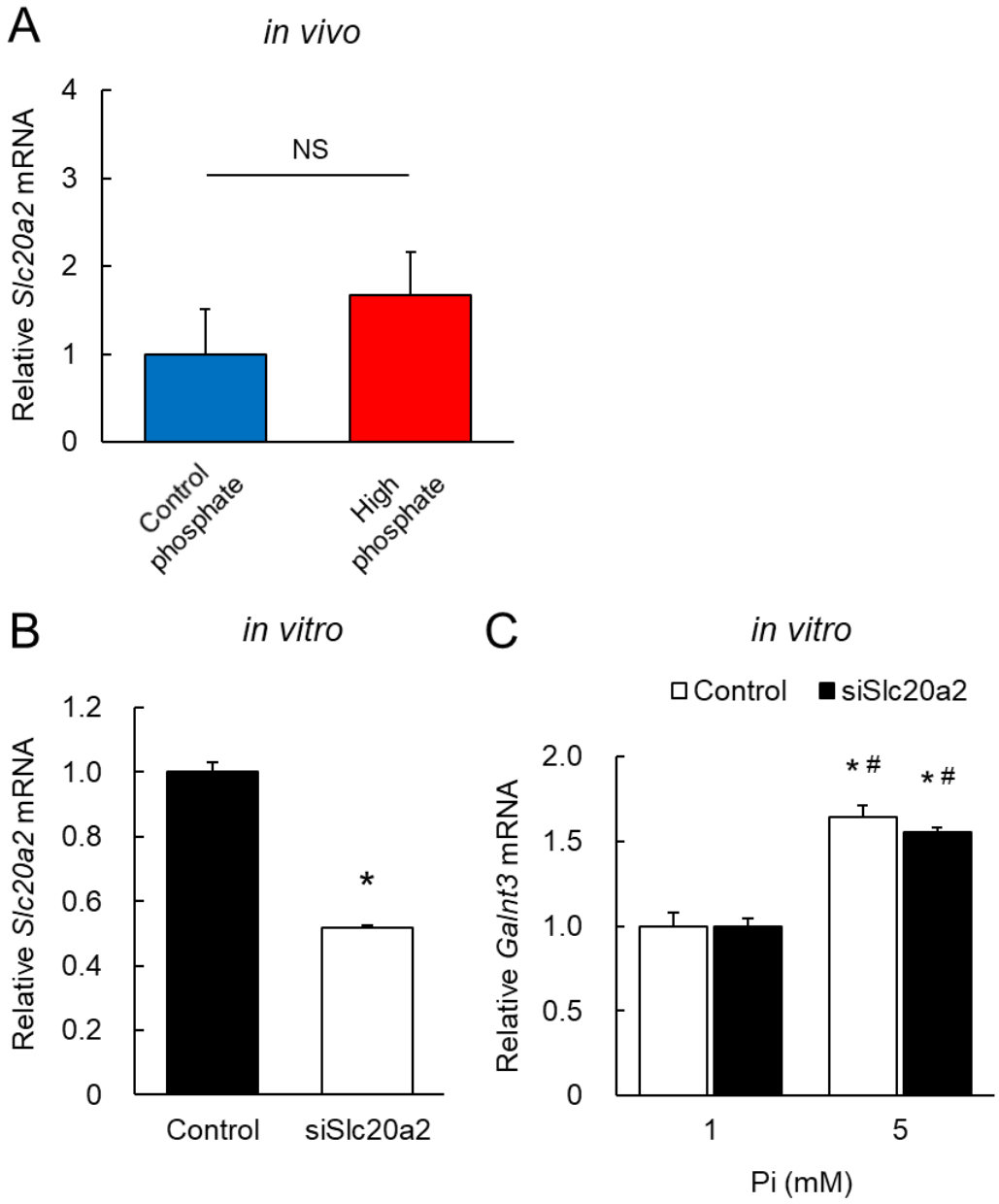

Fig. S10. PiT2 encoded by *Slc20a2* is not involved in the regulation of *Galnt3* by high Pi. (*A*) *Slc20a2* mRNA expression in femurs of mice fed with a CP or HP diet for two weeks. (*B*) siRNA-mediated silencing of *Slc20a2* decreased *Slc20a2* mRNA expression by approximately 50% in UMR106 cells. (*C*) *Galnt3* mRNA expression by 5 mM Pi for 12 hours compared with 1 mM Pi under siRNA-mediated silencing of *Slc20a2*. Data represent the mean±SEM. (*A*) n=19 mice per group; NS: not significant by Student's t-test. (*B*) n=3 per group; *P<0.05 by Student's t-test. (*C*) n=3 per group; *P<0.05 compared to 1 mM Pi without si*Slc20a2*, $^{\#}P$<0.05 compared to 1 mM Pi with si*Slc20a2* by ANOVA with a *post hoc* Tukey's test.

Additional data table S1 (separate file)
Identification and label-free quantification of the peptides with phosphotyrosine in the order of fold increase by high Pi.

Additional data table S2 (separate file)
Targeted quantification using parallel reaction monitoring (PRM).