Increased FGF-23 levels are linked to ineffective erythropoiesis and impaired bone mineralization in myelodysplastic syndromes

Supplementary Materials:

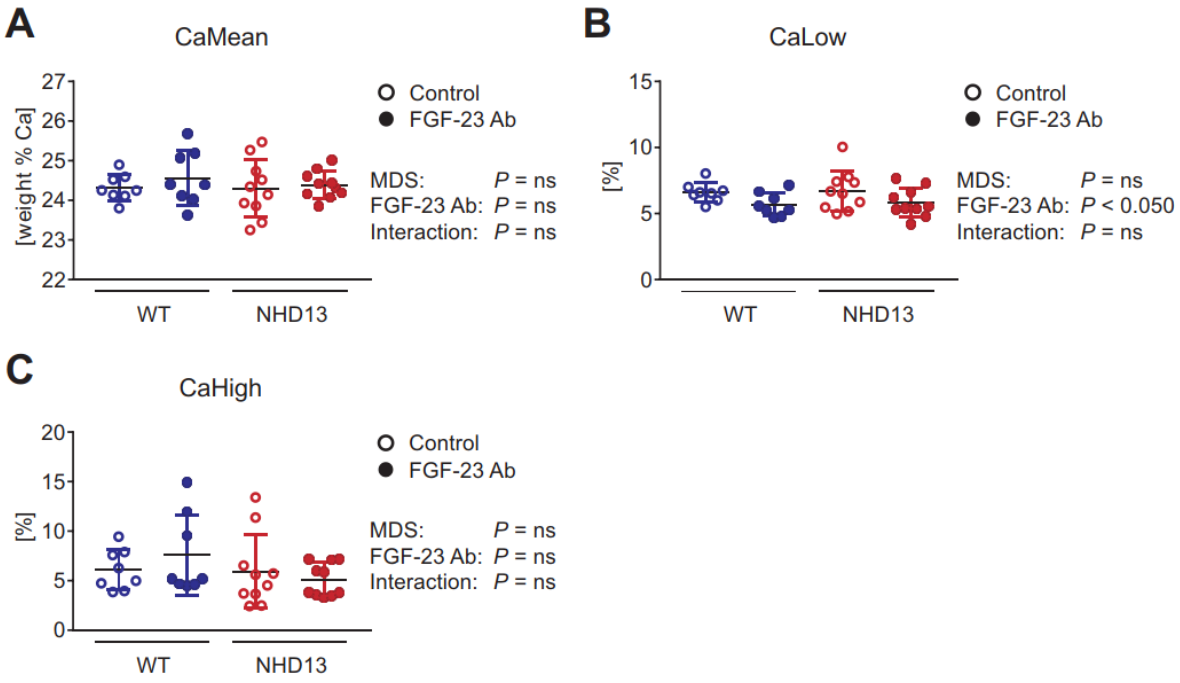

Figure S1. FGF-23 neutralization does not alter calcium concentration in bone of WT and NHD13 mice.

To determine the degree and distribution of bone calcium concentrations after administration of FGF-23 antibody over 8 weeks, tibiae of 4-month-old wild-type (WT) and NUP98/HOXD13 (NHD13) mice were collected. We used quantitative backscattered electron imaging to characterize the mineralized bone matrix by quantifying (A) mean calcium (n=8-10) as well as (B-C) % of lowly (n=8-10) and highly (n=8-10) mineralized area in the tibia. Data are shown as mean ± SD of 5 independent experiments. Statistical analysis was performed by two-way ANOVA for the effect of MDS, FGF 23 antibody treatment, and the interaction of both followed by Bonferroni's multiple comparison.

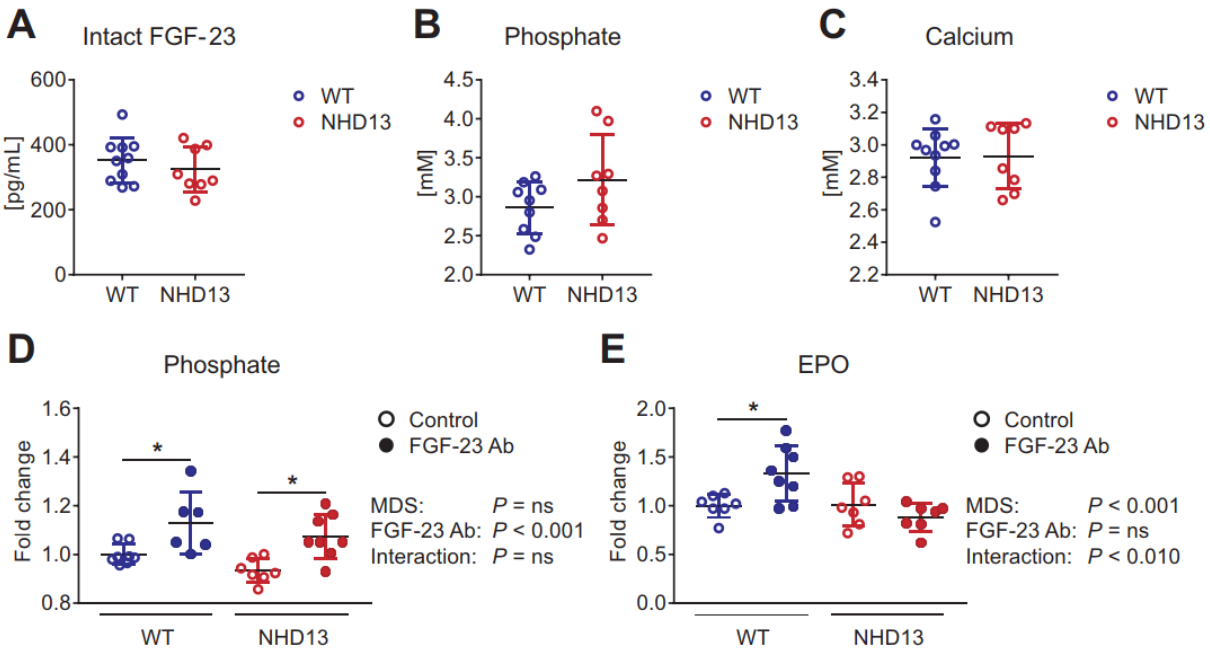

Figure S2. Serum parameters of naïve and FGF-23 antibody treated WT and NHD13 mice.

(A-C) Serum of 6-month-old wild type (WT) and NUP98/HOXD13 (NHD13) mice were collected to determine intact FGF-23 (n=8-10), phosphate (n=8-9), and calcium concentration (n=8-10). (D-E) To assess the effect of FGF-23 antibodies (Ab) on phosphate (n=6-8) and EPO (n=7-8), WT and NHD13 mice were treated with a single injection of FGF-23 antibodies (10 mg/kg). Data are shown as mean ± SD of 3 independent experiments. Statistical analysis was performed by the two-sided Student´s *t*-test (A-C) or by two-way ANOVA for the effect of MDS, FGF-23 antibody treatment, and the interaction of both followed by Bonferroni's multiple comparison (D-E). **P*<0.05 vs. control.

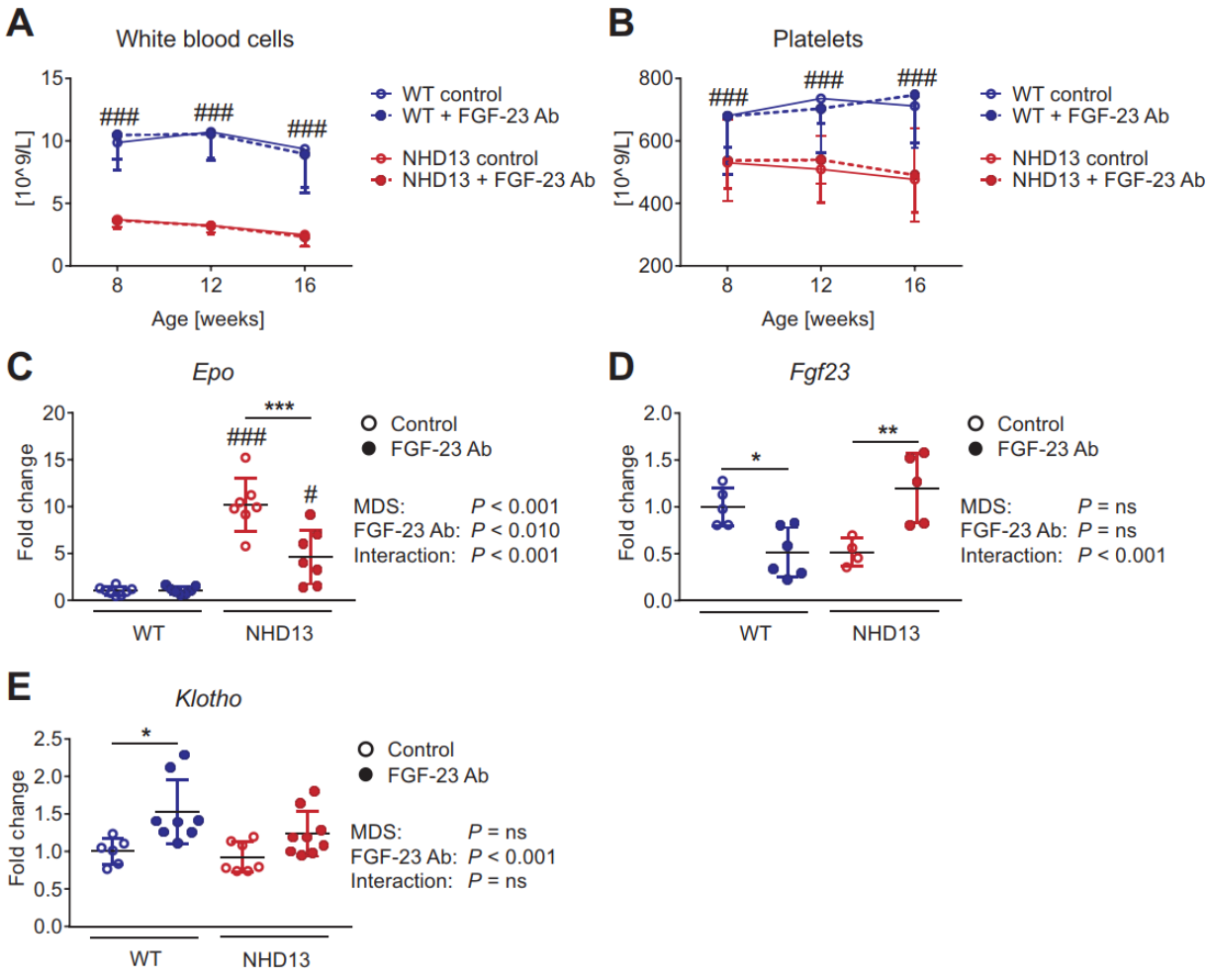

Figure S3. Neutralization of FGF-23 does not prevent leukopenia and thrombocytopenia but regulates *Epo* gene expression in the kidney.

(A-B) Two-month-old wild-type (WT) and NUP98/HOXD13 (NHD13) mice were treated with FGF-23 antibody (Ab) over 8 weeks. Throughout the experiment, retrobulbar blood of the mice was used to analyze white blood cells and platelets in the blood once a month (n=17-19). Data are shown as means. At the end of treatment gene expression of *Epo*, *Fgf23*, and *Klotho* were analyzed in the kidney. Data are shown as mean ± SD of 12 independent experiments. Statistical analysis was performed by two-way ANOVA for the effect of MDS, FGF-23 antibody treatment, and the interaction of both. Statistical significance of Bonferroni multiple comparisons is denoted. **P*<0.05; ***P*<0.01; ****P*<0.001 vs. control. $^{\#}$*P*<0.05; $^{\#\#\#}$*P*<0.001 vs. WT control.

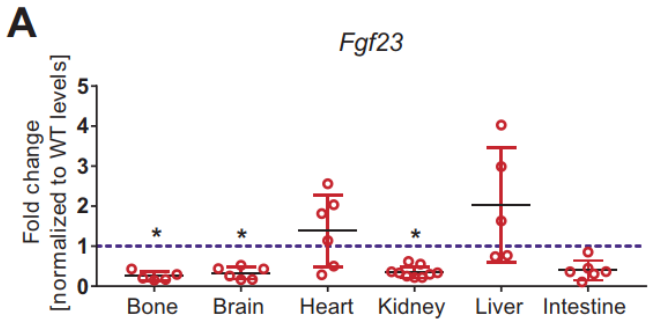

Figure S4. *Fgf23* expression in NHD13 mice organs.

Fgf23 expression was assessed in flushed long bones, brain, heart, kidney, liver, and intestine of 6-month-old wild-type (WT) and NUP98/HOXD13 (NHD13) mice (n=3-8). Dotted line indicates the normalized WT levels. Data are shown as mean ± SD of 3 independent experiments and were analyzed by the two-sided Student´s *t*-test. **P*<0.05 vs. WT mice.

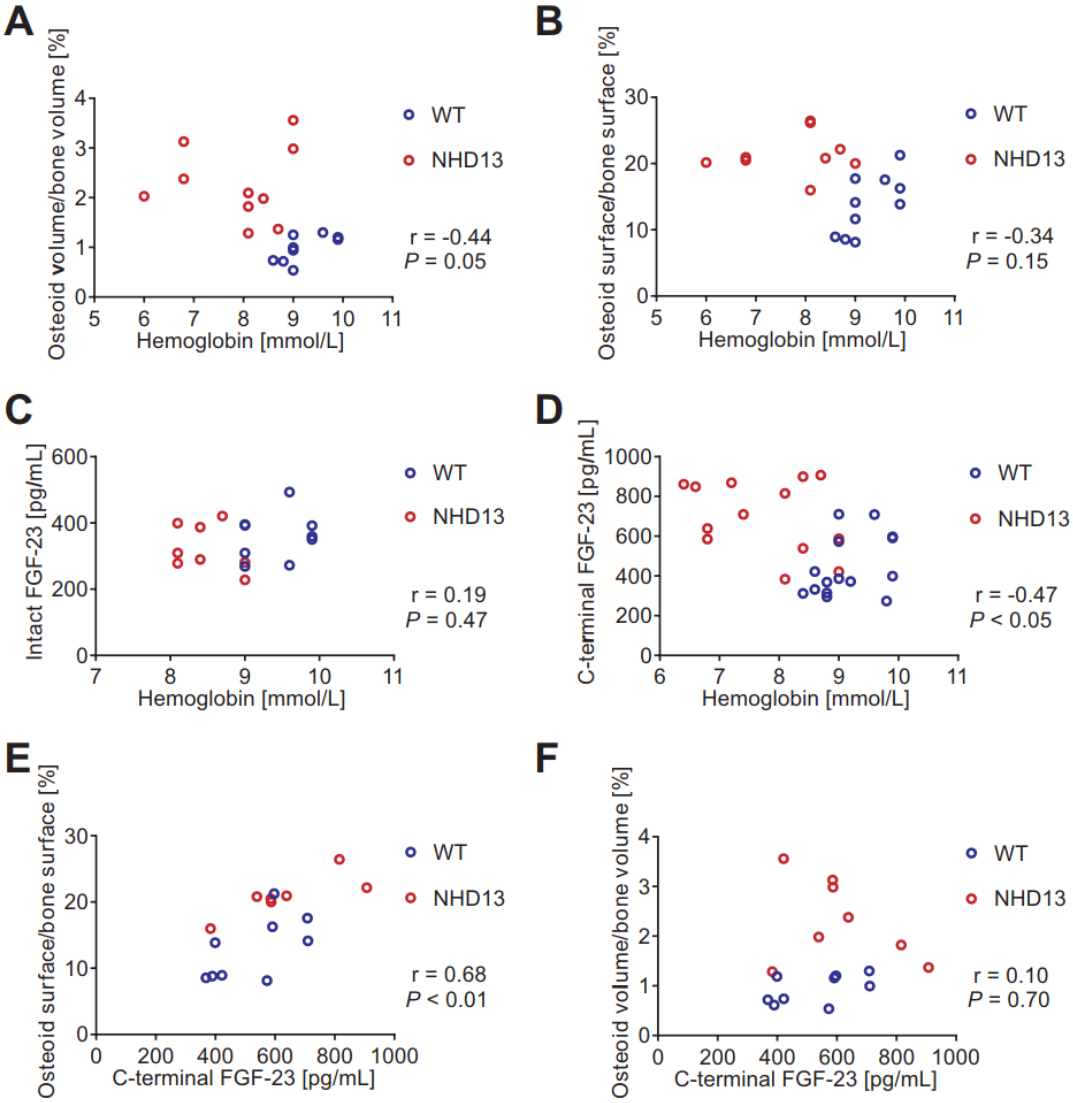

Figure S5. Correlation between FGF-23 and hemoglobin levels or osteoid parameters in NHD13 mice.

Scatter plots and Pearson correlation coefficient (r) were applied to determine the dependence of hemoglobin levels and osteoid volume per bone volume (A, n=21), osteoid surface per bone surface (B, n=19), intact FGF-23 (C, n=17) or C-terminal FGF-23 (D, n=28) as well as the correlation between C-terminal FGF-23 and osteoid surface per bone surface (E, n=16) or osteoid volume per bone volume (F, n=17). In all scatter plots, each dot represents a mouse.

Table S1. Serum parameters of WT and NHD13 mice after 8 weeks of FGF-23 antibody treatment.

	WT		NHD13	
	Control	**FGF-23 Ab**	**Control**	**FGF-23 Ab**
Intact FGF-23 [pg/mL]	364 ± 51.2	5952 ± 272***	324 ± 47.1	6192 ± 174***
C-terminal FGF-23 [pg/mL]	374 ± 56.3	179 ± 26.7***	391 ± 51.5	186 ± 33.0***
PTH [pg/mL]	108 ± 55.0	145 ± 76.2	143 ± 93.6	115 ± 46.7
1,25-(OH)2 D3 [pmol/L]	217 ± 60.5	199 ± 43.8	203 ± 61.5	250 ± 78.4
Calcium [mmol/L]	2.88 ± 0.31	2.93 ± 0.26	2.99 ± 0.18	3.00 ± 0.24
Phosphate [mmol/L]	3.47 ± 0.36	3.78 ± 0.62	3.60 ± 0.28	3.28 ± 0.37
EPO [pg/mL]	843 ± 223	833 ± 199	874 ± 273	831 ± 178

WT, wild-type; NHD13, NUP98/HOXD13; FGF-23 Ab, fibroblast growth factor-23 antibody; PTH, parathyroid hormone; 1,25-(OH)2 D3, 1,25-dihydroxyvitamin D3; EPO, erythropoietin; WT and NHD13 (n=7-10 mice/group). Data are shown as mean ± SD of 5 independent experiments and were analyzed by two-way ANOVA. Statistical significance of multiple comparisons is denoted. ***P<0.001 vs. control. $^{\#}P$<0.05 vs. WT control.