Supplementary Material for:

Knock-in Mouse with mutant Ga11 mimics human inherited hypocalcemia and is rescued by pharmacologic inhibitors

Kelly L Roszko,*[1] Ruiye Bi,*[1,2] Caroline M Gorvin,[3] Hans Bräuner-Osborne,[4] Xiao-Feng Xiong,[4] Asuka Inoue,[5,6] Rajesh V Thakker,[3] Kristian Strømgaard,[4] Thomas Gardella,[1] Michael Mannstadt[1]

A.
Bone Mineral Density by μCT

B.
Bone Volume Fraction by μCT
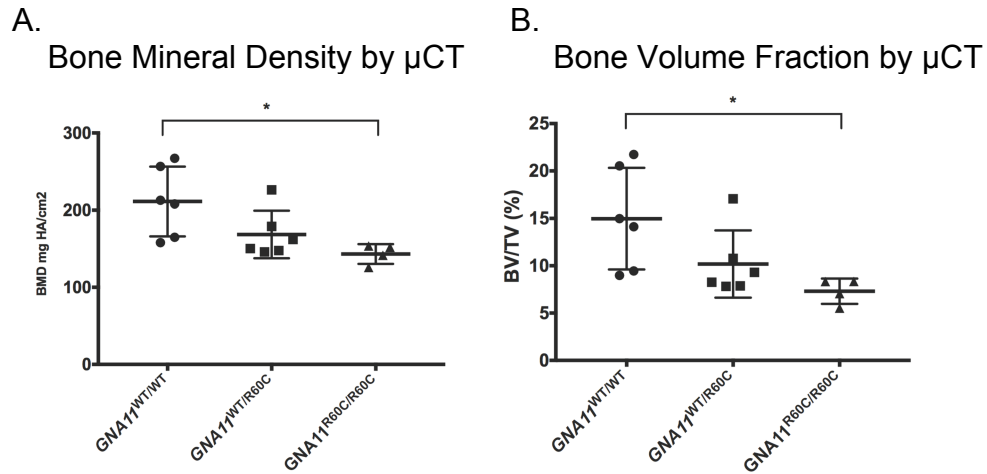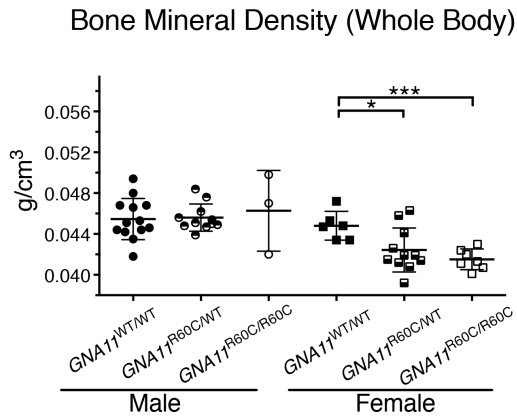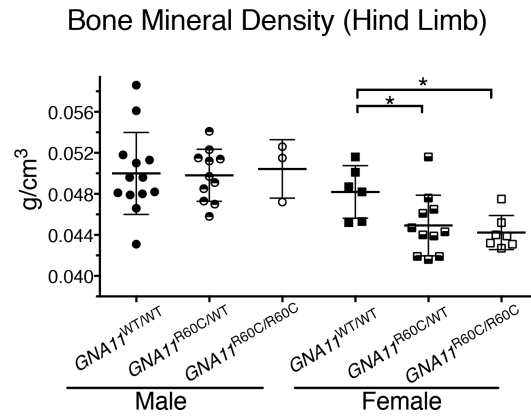

Supplementary Figure 1

Mutant male mice have reduced bone mineral density and bone volume fraction.

μCT analysis revealed significant decreases in bone mineral density (BMD) (A), and bone volume fraction (BV/TV) (B) in 20-week old male $GNA11^{WT/WT}$ (n=6) vs male $GNA11^{R60C/R60C}$ (n=4) mice. There was no significant difference between $GNA11^{WT/WT}$ (n=6) and $GNA11^{WT/R60C}$ (n=6) mice. Analyses were done by ANOVA with Tukey's multiple comparisons. (* p<0.03)

A

Bone Mineral Density (Whole Body)


B

Bone Mineral Density (Hind Limb)

Supplementary Figure 2.

Whole body (A) and hind limb (B) peripheral dual-energy X-ray absorptiometry (pDXA) in 12-13-week old mice. Wild-type ($GNA11^{WT/WT)}$: n=13 males, n=6 females; heterozygous ($GNA11^{R60C/WT}$): n=11 males, n=11 females, and homozygous ($GNA11^{R60C/R60C}$): n=3 males, n=7 females. Data are shown as mean ± SD. Female and male groups were each analysed by ANOVA with Tukey's multiple comparisons tests (*p<0.05, ***p<0.01).
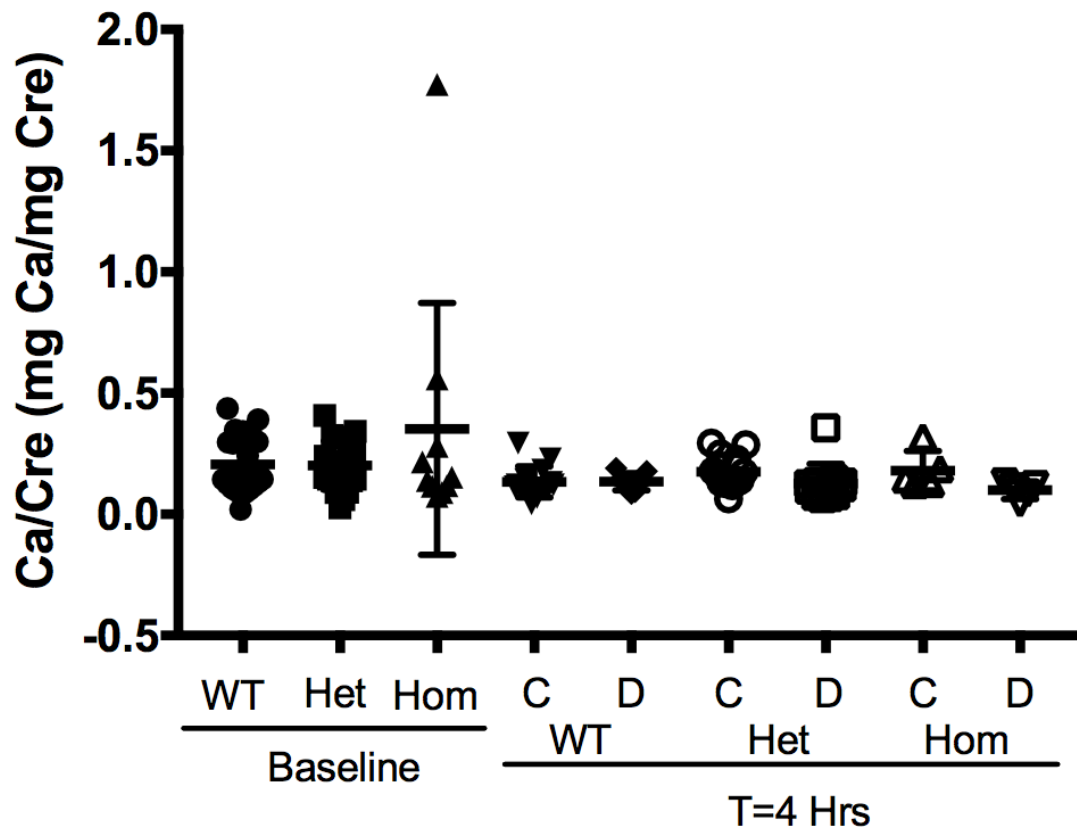
Supplementary Figure 3.

The urinary calcium to creatinine ratio was determined in wild-type ($GNA11^{WT/WT}$, n=28 baseline, n=14 vehicle, n=10 calcilytic), heterozygous ($GNA11^{R60C/WT}$, n=21 baseline, n=13 vehicle, n=10 calcilytic), or homozygous mice ($GNA11^{R60C/R60C}$, n=9 baseline, n=5 vehicle, n=5 calcilytic) at baseline and four hours after bolus intraperitoneal injection of either vehicle (C) or 28 mg/kg of the calcilytic NPS 2143 (D). Data are shown as mean ± SD. There were not significant differences between any of the groups.

	WILD TYPE					GNA11^{R60C/WT}					GNA11^{R60C/R60C}				
	Baseline	4-hours Vehicle	4-hours NPS 2143	24-hours Vehicle	24-hours NPS 2143	Baseline	4-hours Vehicle	4-hours NPS 2143	24-hours Vehicle	24-hours NPS 2143	Baseline	4-hours Vehicle	4-hours NPS 2143	24-hours Vehicle	24-hours NPS 2143
Ionized calcium (mmol/l)	1.25±0.027	1.23±0.021*	1.35±0.10*	1.23±0.029	1.26±0.031	1.20±0.045	1.20±0.045*	1.35±0.093*	1.20±0.039	1.20±0.046	1.12±0.051	1.10±0.04*	1.27±0.04*	1.14±0.046	1.12±0.065
Phosphate (mg/dl)	5.90±1.24	6.0±1.09*	9.06±2.96*	2.74±1.43	2.85±1.15	6.33±1.02	6.40±1.48	8.15±1.42	2.32±1.21	5.65±6.26	7.19±1.11	8.42±1.25	7.70±2.22	4.12±2.71	3.33±0.53
PTH (pg/ml)	211±221	196±103*	2,704±1,816*	190±57	148±53	146±159	207±177*	1,614±976*	138±61	413±674	119±129	362±259*	1,022±581*	179±98	93±30
FeCa (%)	1.72±1.06	1.23±0.60	1.11±0.32			1.91±0.98	1.66±0.66*	0.87±0.25*			2.03±1.68	1.85±0.83*	0.90±0.36*		

Supplementary Table 1. Biochemical parameters of 9-week-old wild-type *GNA11*^{WT/WT}, heterozygous *GNA11*^{R60C/WT}, and homozygous *GNA11*^{R60C/R60C} mice at baseline, four and 24 hours after bolus intraperitoneal injection of either vehicle of the calcilytic NPS 2143. All values are expressed as mean ± SD. Statistical analysis was by Student's *t*-test comparing the vehicle and NPS 2143 groups at a given time point.

* p<0.05 Vehicle vs NPS 2143 treatment.

	WILD-TYPE			GNA11$^{R60C/WT}$			GNA11$^{R60C/R60C}$		
	Baseline	4-hours Vehicle	4-hours YM-254890	Baseline	4-hours Vehicle	4-hours YM-254890	Baseline	4-hours Vehicle	4-hours YM-254890
Ionized calcium (mmol/l)	1.23±0.37	1.22±0.036*	1.31±0.044*	1.20±0.050	1.19±0.062	1.24±0.043	1.10±0.067	1.12±0.032	1.10±0.067
Phosphate (mg/dl)	5.68±0.99	5.14±1.3	4.47±0.97	5.15±1.35	5.22±1.23	5.18±1.25	6.96±1.86	6.02±1.14	6.37±1.57
PTH (pg/ml)	257±230	215±146*	429±280*	121±100	198±196	232±204	108±108	138±89	104±50
FeCa (%)	2.41±2.25	1.67±1.10	0.89±0.58	1.93±0.78	0.65±0.32	0.75±0.18	3.70±2.67	1.78±0.99	1.29±0.36

Supplementary Table 2. Biochemical parameters of 12-week-old wild-type GNA11$^{WT/WT}$, heterozygous GNA11$^{R60C/WT}$, and homozygous GNA11$^{R60C/R60C}$ mice at baseline, four and 24 four hours after bolus intraperitoneal injection of either vehicle or the Gα$_{11/q}$ inhibitor YM-254890. All values are expressed as mean ± SD. Statistical analysis was by Student's *t*-test comparing the vehicle to YM-254890 groups at a given time point. * p<0.05 Vehicle vs YM-254890 treatment.