Supporting Information

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SI Materials and Methods

Gastrin Response to Bombesin. After an overnight fast, mice were injected with bombesin (50 μg/kg body weight; Bachem) as an i.p. bolus. Blood was sampled from the retro-orbital sinus before and 30–45 min after injection.

Gastric Acid Secretion. Acid output was measured by the pylorus ligation method (1). To determine the stimulated acid production, either gastrin-17 (1 mg/kg body weight; Sigma-Aldrich) or histamine dihydrochloride (10 mg/kg body weight; Sigma-Aldrich) was administrated (subcutaneous bolus, 1% wt) immediately after pylorus ligation, and gastric acid was collected at 90 min. Gastric juice was titrated to pH 7.0 with 0.01 N NaOH and expressed as nEq H^+ secreted per hour.

Immunohistochemistry. Stomachs were immersed in 4% (wt/vol) paraformaldehyde in PBS overnight, impregnated with 30% glucose, frozen in Tissue-tech, and sectioned (5 μm). Gastrin was detected using a rabbit anti-human gastrin primary antibody (1:500 dilution overnight; Dako Cytomation) and Alexa 594 goat anti-rabbit secondary antibody. Parietal cell H^+/K^+ ATPase was detected using a mouse monoclonal IgG against amino acid residues 1–13 or 15–28 located on the cytoplasmic side of the β subunit of H^+/K^+ ATPase (1:1,000 dilution; Affinity BioReagents) and detected using Alexa 594 goat anti-mouse IgG (1:500 dilutions; Invitrogen). For double immunoreactivity to calcium-sensing receptor (CaR) and gastrin, stomachs were similarly fixed and paraffin-embedded; 5-μm sections underwent antigen retrieval as described previously (2). The sections were blocked with 3% BSA in PBS blocking buffer for 30 min and then incubated with primary antibodies, including mouse monoclonal anti-human CaR directed at amino acids 214–235 of the extracellular domain of CaR (Affinity Bioreagents) at 1:500 dilution and rabbit polyclonal anti-human gastrin (Dako Cytomation) at 1:500 dilution for 48 h. The sections were then incubated with goat anti-mouse Alexa 488 (Molecular Probes) or goat anti-rabbit Alexa 594 (Molecular Probes) at a dilution of 1:1,000 for 1 h at room temperature. Specimens were analyzed using a Zeiss LSM510 confocal microscope.

1. Shay H, Sun DC, Gruenstein M (1954) A quantitative method for measuring spontaneous gastric secretion in the rat. *Gastroenterology* 26:906–913.

2. Goebel SU, et al. (2000) Expression of the calcium-sensing receptor in gastrinomas. *J Clin Endocrinol Metab* 85:4131–4137.

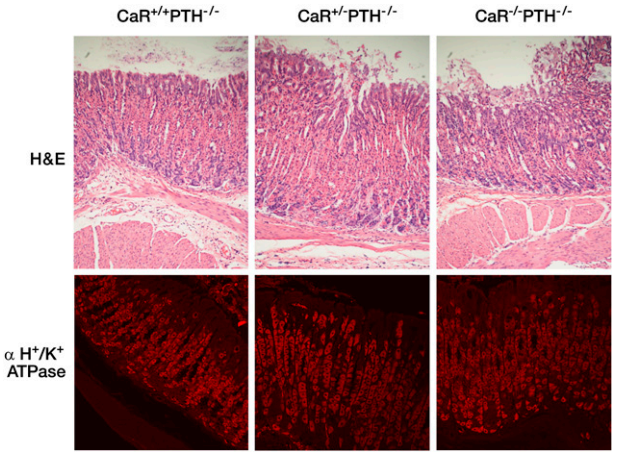

Fig. S1. The gastric bodies of CaR wild type (WT; $CaR^{+/+}PTH^{-/-}$), heterozygous ($CaR^{+/-}PTH^{-/-}$), and null ($CaR^{-/-}PTH^{-/-}$) littermates are histologically indistinguishable. *Upper* shows H&E, and *Lower* is H^+/K^+ ATPase immunohistochemical stains of representative paraffin-embedded and frozen sections, respectively, from the gastric body of mice with the indicated genotypes.

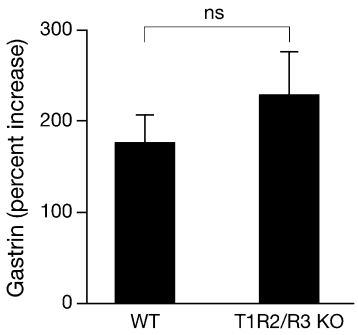

Fig. S2. The sweet T1R2/3 and amino acid T1R1/3 receptor heterodimers do not mediate peptone-stimulated gastrin release. Mice, with homozygous deletions of both T1R2 and T1R3 genes, and their WT littermates were fasted overnight and gavaged with a 1.5% body weight bolus of 8% peptone. Plasma gastrin levels were measured by RIA just before and 30 min after gavage. Results are expressed as the percent change (mean ± SE) in secretion relative to basal secretion (n = 8 mice). ns, nonsignificant.

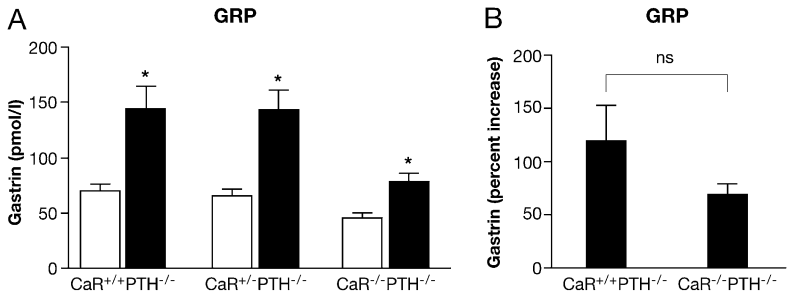

Fig. S3. Bombesin significantly stimulates gastrin secretion in CaR WT ($CaR^{+/+}$ $PTH^{-/-}$) and null ($CaR^{-/-}$ $PTH^{-/-}$) littermates. (*A*) Mice, fasted overnight, were s.c. injected the following morning with the amphibian homolog of gastrin-releasing peptide, bombesin (50 μg/kg). Plasma gastrin was measured by RIA just before (open bars) and 30 min after injection (filled bar). (*B*) The same data are presented as percent increase over basal. *$P < 0.05$, stimulated vs. basal (n = 10 mice). ns, nonsignificant.

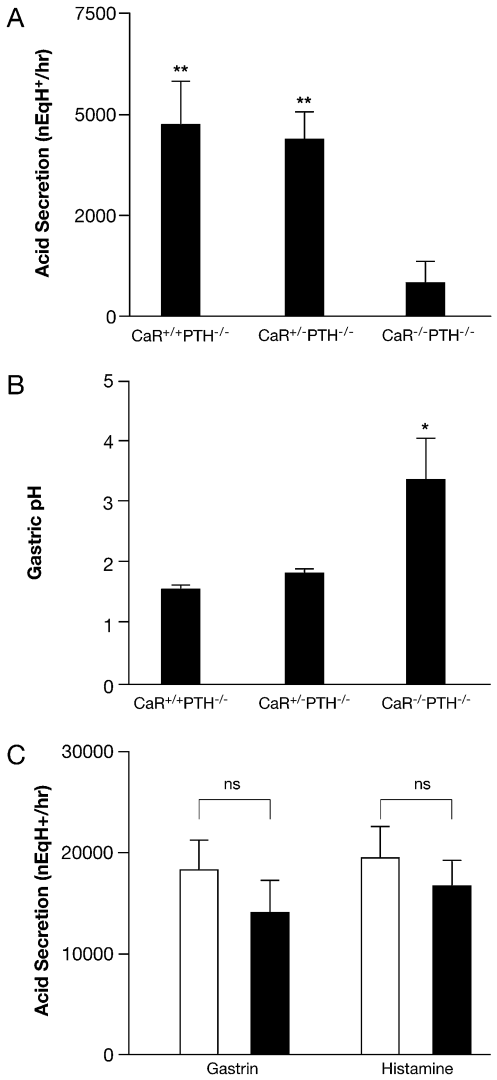

Fig. S4. CaR-null ($CaR^{-/-}PTH^{-/-}$) mice have markedly reduced basal gastric acid secretion and elevated gastric secretion pH compared with WT ($CaR^{+/+}PTH^{-/-}$) littermates but an equivalent gastric acid secretory response to gastrin and histamine. (*A*) Basal gastric acid secretion ($n = 6$ mice). $^{**}P < 0.01$ relative to CaR-null ($CaR^{-/-}PTH^{-/-}$) littermates. (*B*) pH of luminal gastric secretion in pylorus-ligated stomachs from mice with the indicated CaR genotypes ($n = 6$ mice). $^{*}P < 0.05$ relative to both $CaR^{+/+}PTH^{-/-}$ and $CaR^{+/-}PTH^{-/-}$ littermates. (*C*) Gastrin and histamine-stimulated gastric acid secretion in WT ($CaR^{+/+}PTH^{-/-}$; open bars) vs. null ($CaR^{-/-}PTH^{-/-}$; closed bars) littermates ($n = 6$ mice). ns, nonsignificant.

Table S1. High Ca^{2+} diet does not significantly elevate plasma Ca^{2+} regardless of CaR genotype

	Genotype			
Diet	$CaR^{-/-}PTH^{-/-}$	$CaR^{+/-}PTH^{-/-}$	$CaR^{+/+}PTH^{-/-}$	$CaR^{+/+}PTH^{+/+}$
Normal Ca^{2+} diet (rat chow) Ca^{2+} (total plasma, mmol/L)	1.66 ± 0.04	1.64 ± 0.05	1.72 ± 0.07	2.35 ± 0.02*
High Ca^{2+} diet Ca^{2+} (total plasma, mmol/L)	1.76 ± 0.09	1.68 ± 0.05	1.71 ± 0.05	NA
Fasting gastrin (pmol/L)	43 ± 5†	86 ± 8	63 ± 17	64 ± 9
Meal-stimulated gastrin (pmol/L)	44 ± 5†	161 ± 24	175 ± 21	180 ± 85

The CaR-null ($CaR^{-/-}PTH^{-/-}$) genotype but not total plasma Ca^{2+} determines the gastrin response to meal stimulation. Mice were maintained on a high Ca^{2+} and vitamin D diet for 2 mo. Meal stimulation consisted of ad libitum normal rat chow ($n = 4$ mice/group; data are presented as the mean ± SE).

$^{*}P < 0.01$; $CaR^{+/+}PTH^{+/+}$ vs. $CaR^{+/+}PTH^{-/-}$, $CaR^{+/-}PTH^{-/-}$, or $CaR^{-/-}PTH^{-/-}$.

$^{\dagger}P < 0.01$; $CaR^{-/-}PTH^{-/-}$ vs. $CaR^{+/+}PTH^{+/+}$, $CaR^{+/+}PTH^{-/-}$, or $CaR^{+/-}PTH^{-/-}$.