

Sections from wild-type mouse gastric wall double-stained for (A-C) VAChT and GRP, (D-F) VAChT and VIP, (G-I) GFR α 2 and VAChT, and (J-L) GFR α 2 and TH. A-C: Most if not all cholinergic fibers (A, green) in the mucosa appear to contain GRP (B, magenta). Colocalization is seen as white in the merged image (C). D-F: Most of the VIP-positive fibers (E, magenta) colocalize with VAChT (D, green). G-I: A section from wild-type mouse gastric mucosa double-stained for GFR α 2 and VAChT. Many GFR α 2-positive (G, green) fibers in the mucosa are also labeled with VAChT (H, magenta). The arrow (I) indicates a GFR α 2-positive glial cell associated with VAChT-positive cholinergic nerve fibers. J-L: GFR α 2 (J, green) is not expressed in the TH-positive sympathetic nerve fibers (K, magenta) that innervate a blood vessel at the border of mucosa and submucosa (L, arrow).



Sections through the oxyntic part of gastric mucosa from adult wild-type (WT, A and D)) and GFR α 2-KO (B and E) mice immunostained for GRP (A-B) and galanin (D-E). (C) The density of GRP-positive nerve fibers in the gastric mucosa is reduced by >90% in the KO mice. **, P = 0.015 between the genotypes using t-test, n = 3 mice in both groups. Galanin-positive nerve fibers are abundant in the muscle layers in both genotypes but only few can be found in the mucosa (arrowhead).



In situ hybridization of adjacent stomach sections from postnatal day 14 (P14) mouse shows that neurturin (*Nrtn*) mRNA is selectively expressed in the basal half of the gastric mucosa but not in the muscle layers. In contrast, *Gdnf* mRNA is not expressed in the gastric mucosa. Corresponding bright-field images (right) of the dark-field images (left) muc; mucosa.



Effect of pentagastrin on gastric acid output in fasted wild-type (WT) and GFRa2-KO mice without anesthesia. Pentagastrin (0.5 mg/kg) or saline were administered subcutaneously 40 min before sacrifice and analysis of gastric acid content. Pentagastrin stimulated acid secretion in wild-type mice (# P = 0.003 compared to saline) but not in the GFRa2-KO mice (P = 0.12 compared to saline). ** P = 0.025 between the genotypes. Statistical analysis was done using two-way ANOVA followed by Student-Newman-Keuls test. Values are mean +/- SEM. Number of animals per group is shown in parenthesis.



Deficient cholinergic innervation of the gastric mucosa in 2week-old GFR α 2-KO mice. Sections through the oxyntic part of stomach from postnatal day P14 wild-type and KO littermate mice were immunostained for VAChT.



GFR α 2-KO mice have apparently normal number of Schwann cells in the sublingual salivary and lacrimal (Hadrenian) glands. Sections from adult wild-type (WT) and KO mice were double-stained for GFR α 2 and the Schwann cell marker S100 β .