Supplementary Figures and Tables

Figure S1.

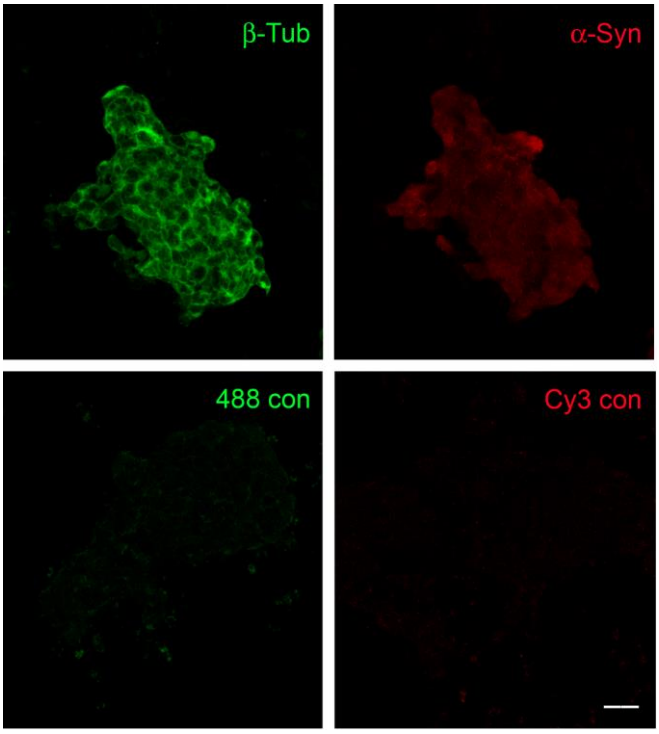

Figure S1. Specificity of immunofluorescence staining in STC-1 cells. STC-1 cells were treated with donkey-anti rabbit antibody conjugated with Dylight488 or donkey anti-mouse antibody conjugated with Cy3 in the absence of the corresponding primary antibody. Note the lack of fluorescence in images without primary antibody. Images were collected at the same gain as cells treated with primary and secondary antibodies. Scale bar 20 μm.

Figure S2.

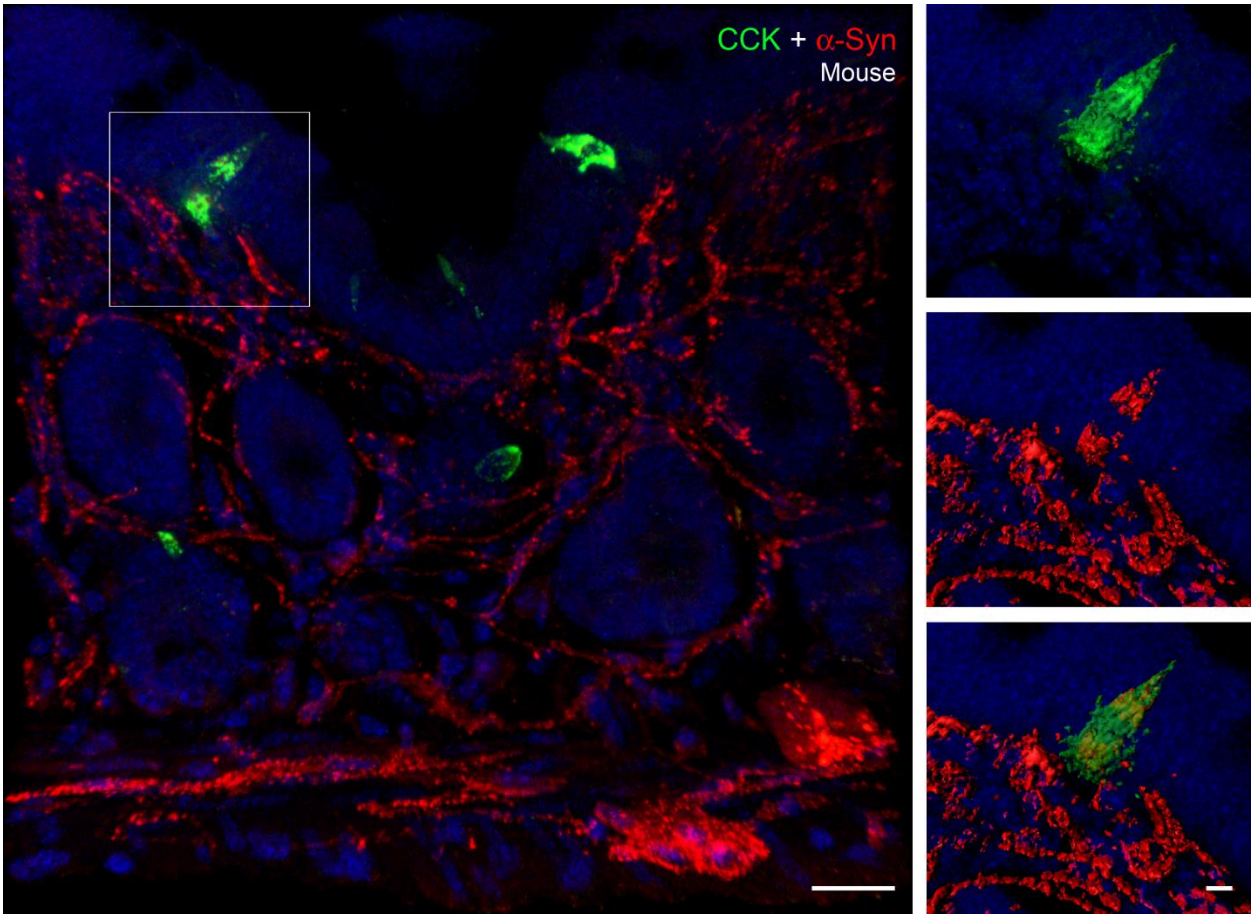

Figure S2. Alpha-synuclein is present in CCK cells and enteric nerves of A53T mouse duodenum. Frozen sections (10 μm thickness) of A53T mouse duodenum showing the crypt region and submucosal ganglia. Alpha-synuclein (red) is present in the cytoplasm of a CCK cell (green) which has been rendered 50% transparent (lower right panel). Alpha-synuclein is also expressed in enteric nerves and sub-mucosal ganglia. Scale bar 20 μm left panel, 5 μm right panels.

Figure S3.

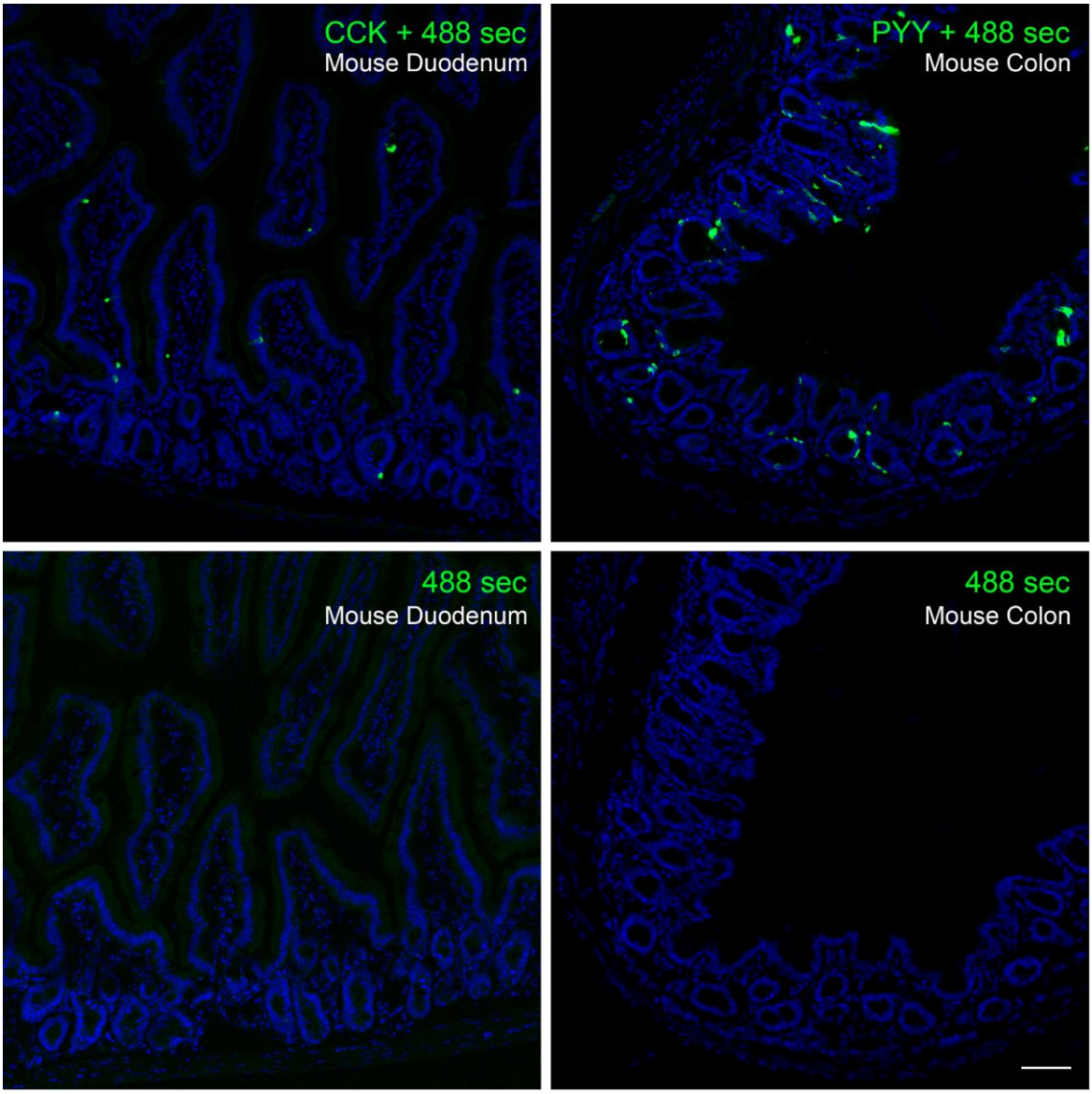

Figure S3. CCK and PYY immunostaining of mouse intestine. Mouse duodenum and colon sections were incubated with CCK and PYY primary antibodies respectively (top panel) along with Alexa Fluor® 488 conjugated donkey anti-rabbit secondary antibody (488 sec), or secondary antibody alone (bottom panel). Sections were imaged at the same gain using a 20X objective. No fluorescence was observed in the absence of primary antibody. Scale bar 50 μm.

Figure S4.

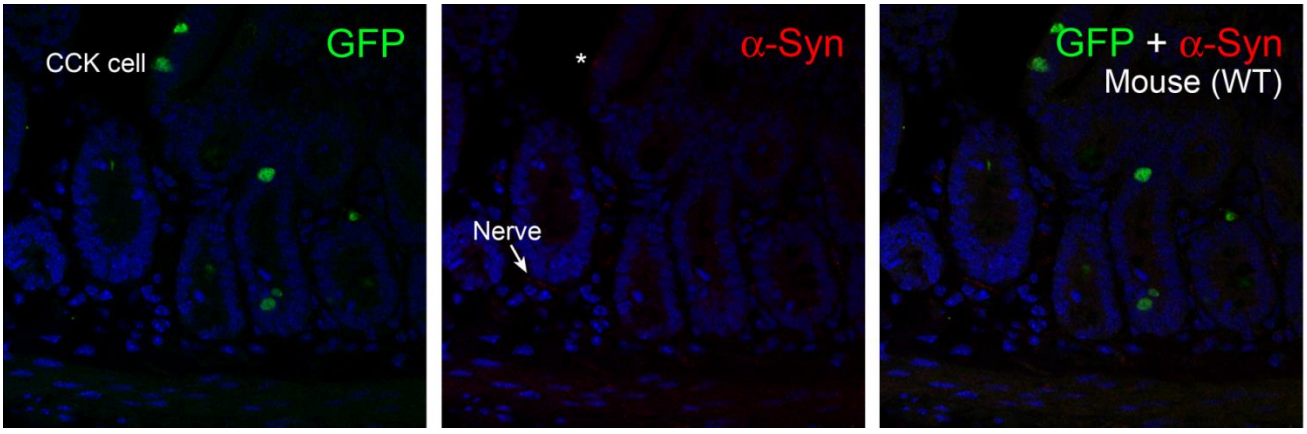

Figure S4. Alpha-synuclein immunofluorescence in wild type mice. Frozen sections (10 μm thickness) of CCK-GFP mouse duodenum were fixed with a mixture of methanol and alcohol and exposed to primary antibodies for GFP (green) and α-synuclein (red). Alpha-synuclein immunofluorescence is faintly visible in the basolateral surface of a CCK cell (marked with asterisk) and in enteric nerves.

Figure S5.

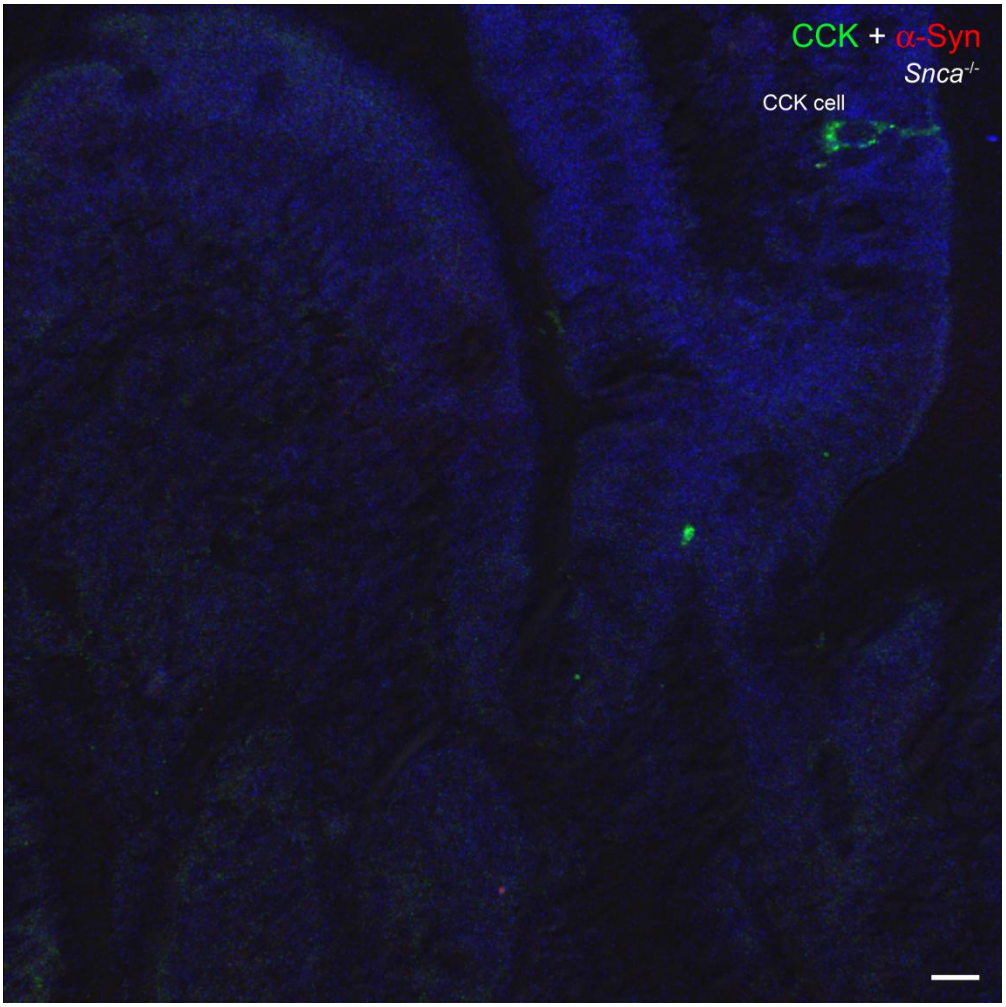

Figure S5. Alpha-synuclein immunofluorescence in *Snca*$^{-/-}$ mice. Frozen sections (10 μm thickness) of *Snca*$^{-/-}$ mouse duodenum were fixed with a mixture of methanol and alcohol and exposed to primary antibodies for CCK (green) and α-synuclein (red). No staining was visible in the red channel. Scale bar 10 μm.

Figure S6.

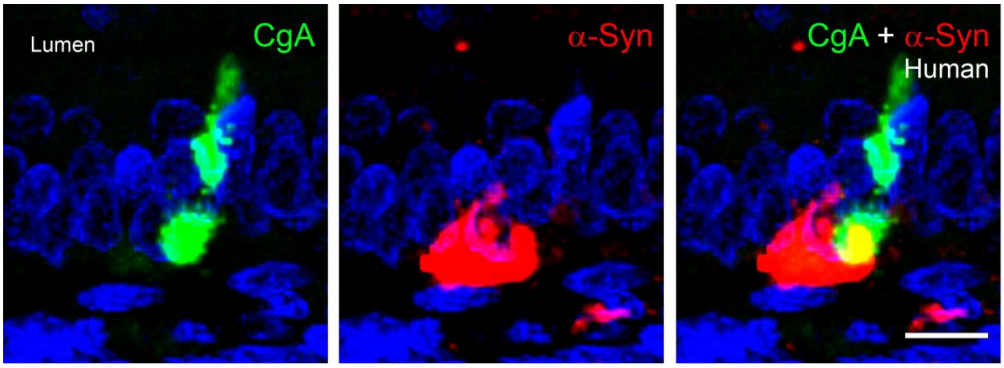

Figure S6. Alpha-synuclein is expressed in chromogranin A positive EECs. Paraffin-embedded section (5 μm thickness) of human duodenum showing chromogranin A (CgA) (green) positive mucosal cell with α-synuclein (red) immunofluorescence in the basolateral region. Scale bar 10 μm.

Figure S7

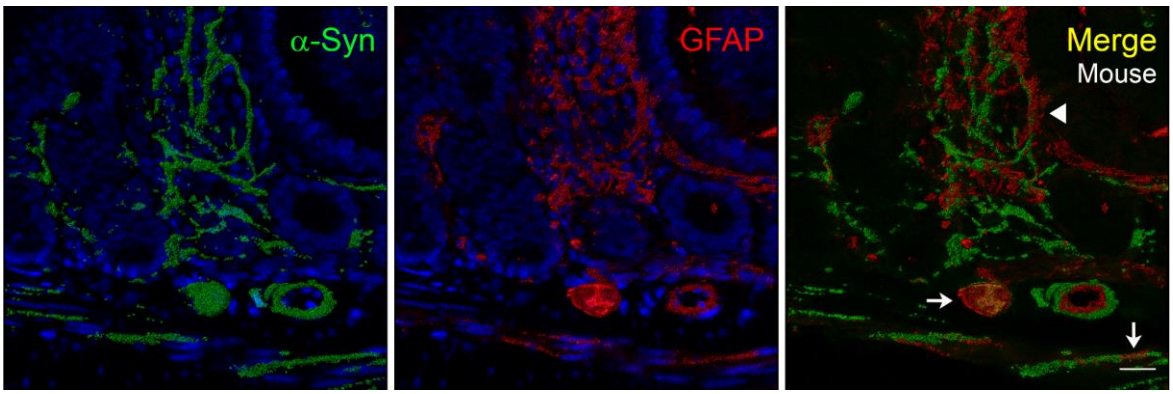

Figure S7. Alpha-synuclein and GFAP immunostaining in mouse duodenum.

Frozen section (16 μm thickness) of A53T mouse duodenum showing α-synuclein (green) and GFAP (red, chick GFAP antibody) immunofluorescence. In certain regions α-synuclein appeared to colocalize with GFAP (orange voxels marked with arrows). However, in the majority of regions where α-synuclein and GFAP were present in close proximity, GFAP immunostaining appeared to wrap around the α-synuclein stained nerves (arrowhead). Scale bar 20 μm.

Table S1. List of primary antibodies used for immunoblotting and immunofluorescence.

Antigen	Source	Catalog #	Species	Dilution
Chromogranin A (CgA)	Abcam	ab15160	Rabbit	1:1000
Cholecystokinin (CCK)	Custom, aa 19-36	Chandra et al., 2010	Rabbit	1:1000
Glial Acidic fibrillary protein (GFAP)	EMD Millipore	AB5541	Chick	1:100
Glial Acidic fibrillary protein (GFAP)	Abcam	ab53554	Goat	1:500
Green fluorescent protein (GFP)	Abcam	ab13970	Chick	1:1000
Peptide YY (PYY)	Custom, aa 4-21	Bohórquez et al., 2011	Rabbit	1:1000
Protein gene product 9.5 (PGP9.5)	EMD Millipore	AB1761-I	Rabbit	1:50
α-Synuclein (α-Syn)	BD Transduction Laboratories	610787	Mouse	1:1000
α-Synuclein (α-Syn)	Abcam	ab6162	Sheep	1:1000
β-Tubulin (β-Tub)	Novus Biologicals	NBP-1-57005	Rabbit	1:1000
Tyrosine hydroxylase (TH)	Aves Labs Inc.	TYH	Chick	1:500

Secondary antibody, Source: Jackson ImmunoResearch Laboratories, Inc.	**Cat#**
Alexa Fluor® 488-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti- Chicken IgY (IgG) (H+L)	703-546-155
Cy™3-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Chicken IgY (IgG) (H+L)	703-166-155
Alexa Fluor® 647-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti- Chicken IgY (IgG) (H+L)	703-606-155
Alexa Fluor® 488-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Goat IgG (H+L)	705-546-147
Cy™3-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Mouse IgG (H+L)	715-166-150
Alexa Fluor® 488-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Rabbit IgG (H+L)	711-546-152
DyLight® 488-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Rabbit IgG (H+L)	Discontinued
Cy™3-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Rabbit IgG (H+L)	711-166-150
Alexa Fluor® 488-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Sheep IgG (H+L)	713-546-147
Cy™3-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Sheep IgG (H+L)	713-166-147
Alexa Fluor® 647-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Sheep IgG (H+L)	713-606-147
Peroxidase-conjugated AffiniPure F(ab')$_2$ Fragment Donkey Anti-Sheep IgG (H+L)	713-036-147

Table S2