

Supplementary Figure 1. Sox10::Cre;R26ReYFP transgenic line was used to lineally mark the progeny of Sox10-expressing cells in the ENS.

(A) Laser confocal microscope image of a whole mount gut preparation from an E12.5 Sox10::Cre;R26ReYFP embryo immunostained for YFP. (B) Short-term cultures of dissociated gut from E16.5 embryos of the same genotype were immunostained for YFP (green) and Sox10 (red). Most Sox10⁺ cells undergo Cre-mediated recombination and, therefore, express YFP (arrows). Arrowhead indicates a Sox10⁺ cell that does not express YFP. (C) Quantification of Sox10⁺ cells that undergo Cre-mediated recombination and, therefore, express YFP. Graph error bars, s.e.m. Oe, oesophagus; St, stomach; Mg, midgut; Ce, caecum; Hg, hindgut. Scale bar, 30 µm.



Supplementary Figure 2. YFP is appropriately expressed in the Sox10expressing cells and their derivatives in Sox10::iCreER^{T2};R26ReYFP transgenics.

(A-B) YFP immunostaining of E12.5 $Sox10::iCreER^{T2};R26ReYFP$ transgenic embryos exposed to 4-OHT at E9.5. YFP expression was detected in DRG, nerve fibers sprouting from the spinal cord area **(A)** and the radial and ulnar nerves of the limb **(B)**. **(C-D)** YFP immunostaining of coronal sections of E12.5 $Sox10::iCreER^{T2};R26ReYFP$ transgenic embryos exposed to 4-OHT at E9.5. YFP expression was detected in the cranial ganglia **(C)** and heart **(D)**. Scale bars, 200 μ m **(A, B)**; 100 μ m **(C, D)**.



Supplementary Figure 3. YFP^+ glial cells from the ENS of hGFAP:: $iCreER^{T2}$; R26ReYFP transgenics generate neurons in culture.

(A) $hGFAP::iCreER^{T2}; R26ReYFP$ transgenic mice were treated with 4-OHT (0.2mg/g) at P84 and analised at P140. Immunostaining of MS-MP whole mount preparations for YFP (green), HuC/D (red) and S100 β (blue) showed that YFP expression is restricted to the S100 β^+ population (arrowheads in inset of panel A). No YFP⁺HuC/D⁺ cells were found. Arrows and diamond arrows in insets of panel A point to YFP⁻HuC/D⁺S100 β^- and YFP⁻HuC/D⁻S100 β^- , respectively. (B) Quantification of the fraction of YFP⁺ cells in the S100 β^+ population. The error bars indicate s.e.m. (C-E) Cell cultures from dissociated MS-MP strips were immunostained for YFP (green), the pan-neuronal marker Tuj1 (red, C) or the neuronal subtype marker nNOS (red, D) and NPY (red, E). Scale bars, 60 µm (A); 10 µm (C-E).