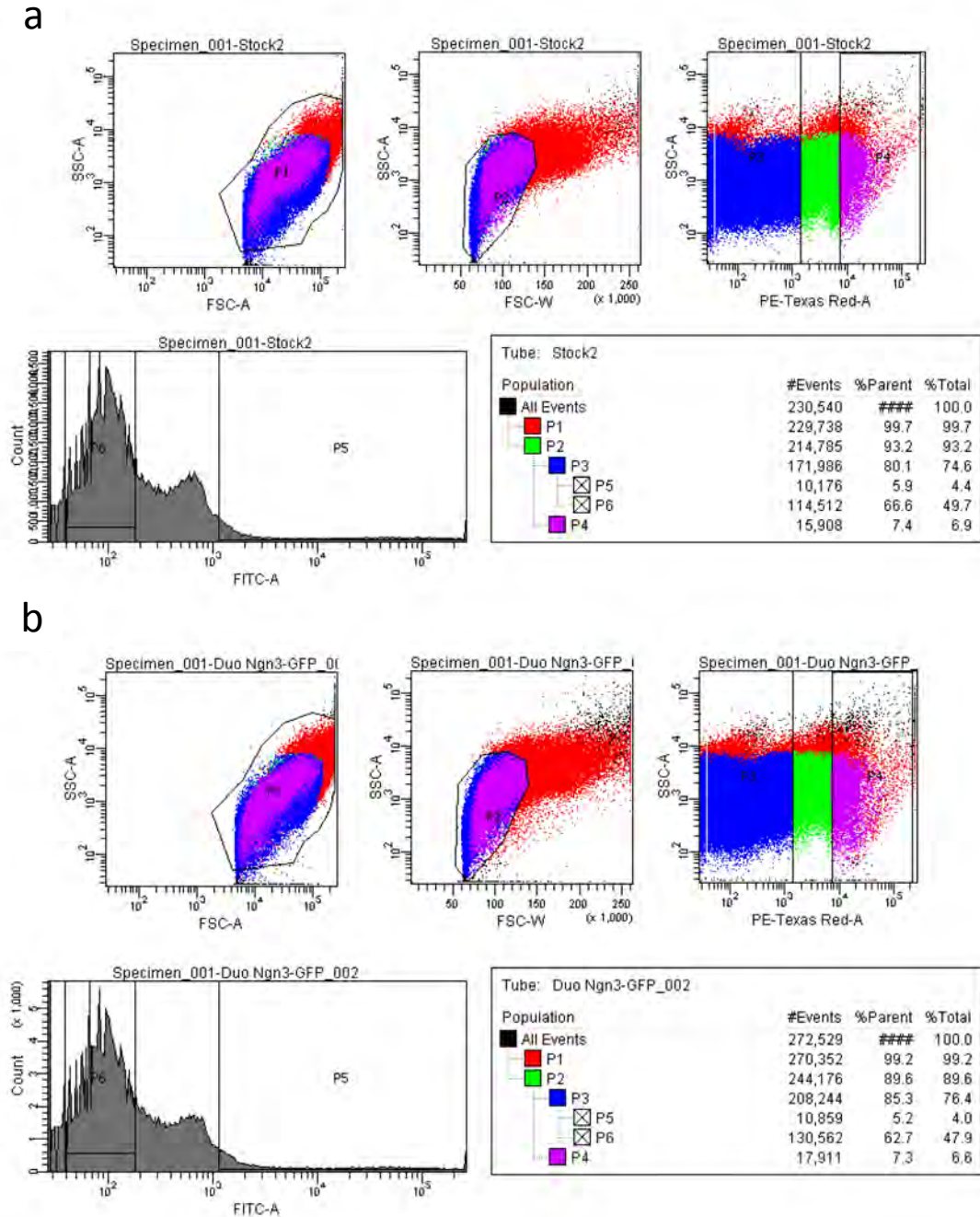


Supplementary Figure 1

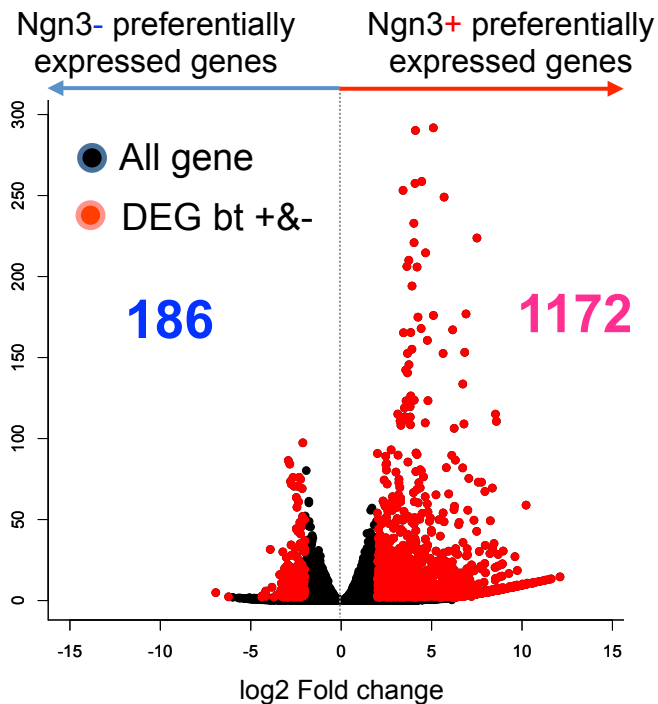
Merged images from Fig. 2 with their single fluorescent images. Scale bar: 50 μ m



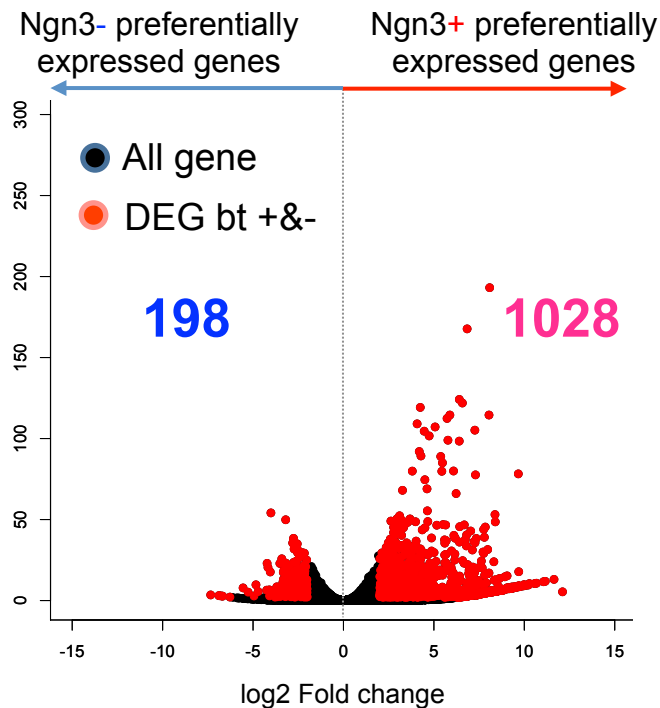
Supplementary Figure 2

Ngn3-EGFP-marked cell sorting of intestinal organoids from jejunum (a) and duodenum (b). All cell populations were initially gated through a forward scatter area (FSC-A) versus side scatter area (SSC-A). Doublets/multiplets and dead cells were eliminated by P2-P4 gating and subsequently singlet live cells (P3) were gated based on Ngn3-EGFP expression. Only 4.0%–4.4% of total cells from organoids was purified as EGFP-positive cells.

Duodenum



Jejunum



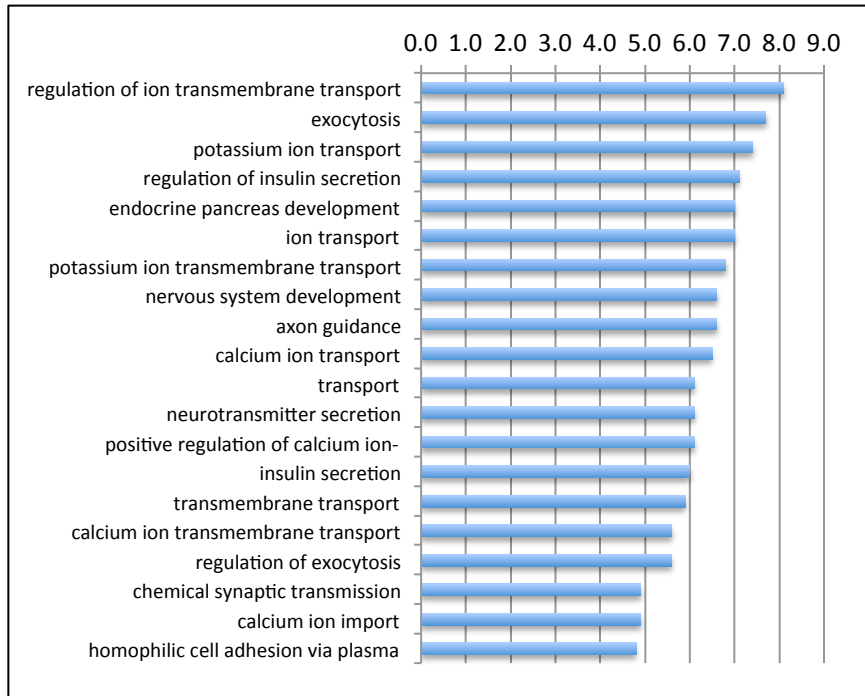
Supplementary Figure 3 (Modified volcano plot analysis from Figure 3)

Ngn3+ preferentially expressed and non-expressed genes were marked in red.

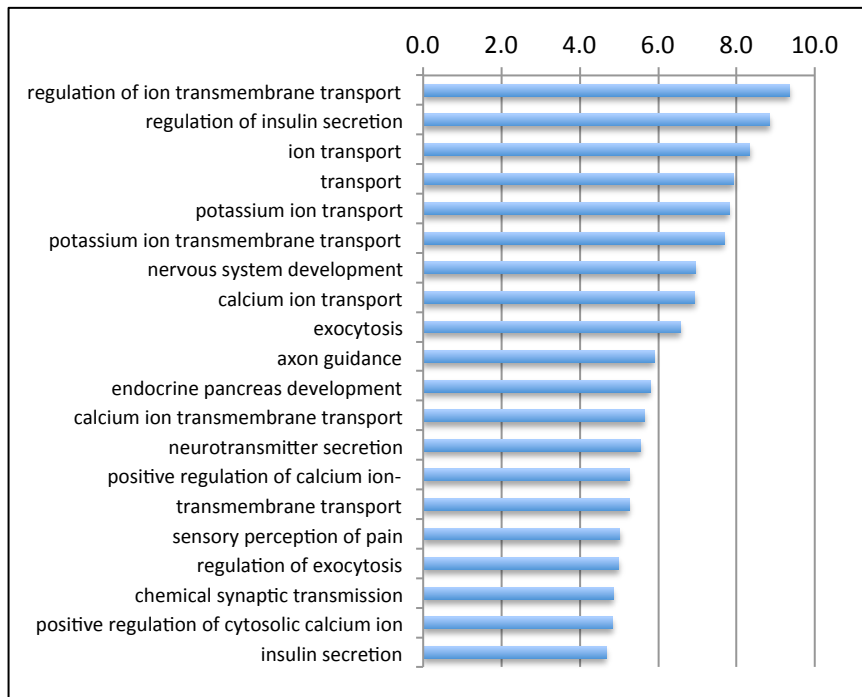
(Fold change ≥ 2 , *padj* ≤ 0.01 , Binominal (Wald) test with Benjamin-Hochberg correction).

$-\log_{10}(p \text{ value})$

Duodenum



Jejunum



GO terms enriched in GFP+ fraction

Supplementary Figure 4

The top 20 GO terms from the preferentially expressed genes screened from the Ngn3+ cell population. $-\log_{10}(P \text{ value})$ is the negative log10 of the P value.

RT-PCR primer

| Gene | Forward 5'→3' | Reverse 5'→3' |
|--------------|----------------------|------------------------|
| <i>G3pdh</i> | ACCACAGTCCATGCCATCAC | TCCACCACCCTGTTGCTGTA |
| <i>Ghrl</i> | GACAAGCAGAAGAGACAGAG | TCTTCCCAGAGGATATCCTG |
| <i>Cck</i> | TAGCGCGATACATCCAGCAG | GGTCACTTATTCTATGGCTGGG |
| <i>Gip</i> | CCTGCTCTCTGTTGCTGGTG | CTGCGTACCTTGGACCTC |
| <i>Sct</i> | CGAACACTCAGACCCTACAG | CAGTCCACTCTGAACGATCAA |
| <i>Gcg</i> | GCTTATAATGCTGGTGCAAG | TTCATCTCATCAGGGTCCTC |

Supplementary Table 1

Primer sets used for RT-PCR in the study.