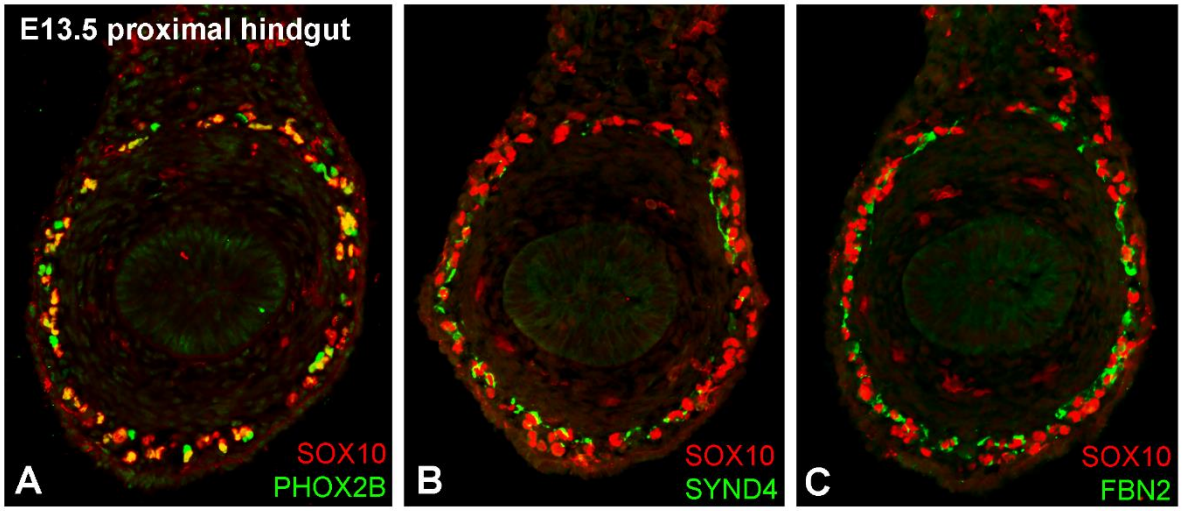

Fig. S1. Immunohistochemistry of SOX10, PHOX2B, FBN2 and SYND4 at 13.5 dpc. Consecutive transverse sections of 13.5 dpc mouse mid-hindgut stained for SOX10 and PHOX2B to mark the ENCDCs **(A)**. FBN2 **(B)** and SYND4 **(C)** expression is present only occasionally around SOX10+ ENCDCs.

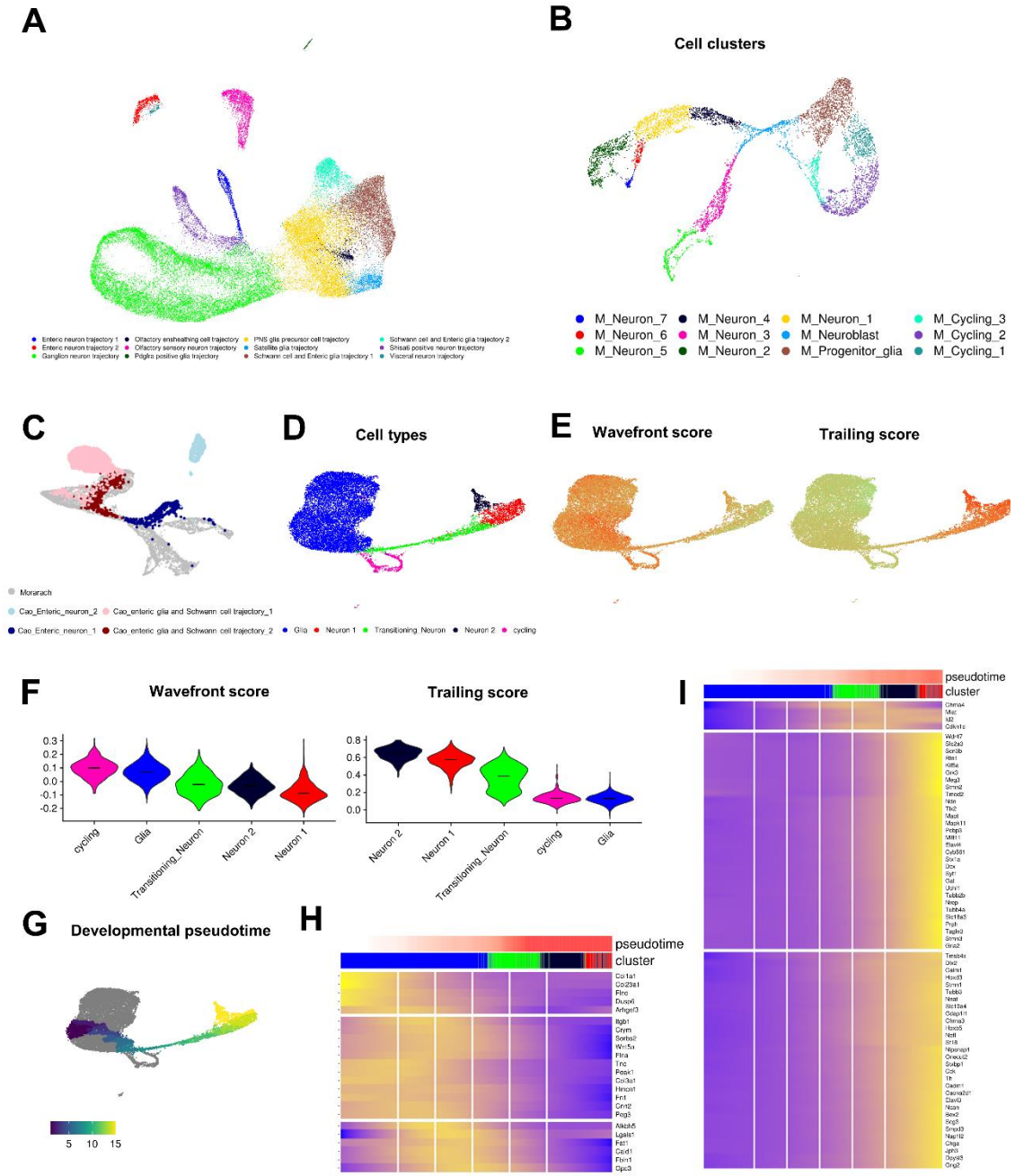

Fig. S2. Supplemental scRNA-seq analysis in embryonic and postnatal ENCDCs. A) UMAP plot of original authors annotations for peripheral nervous system (PNS) neural crest cell trajectories refined by cluster from single cell RNA-seq data originally generated by Cao et al. (2019). B) Unsupervised clustering of the Morarach et al dataset containing Wnt1-tdT expressing ENCDCs at 15.5 and 18.5 dpc represented as UMAP. C) UMAP visualization of cell type annotations from single cell RNA-seq data from Plp^{GFP} cell of enteric neurospheres derived from the postnatal small bowel produced by *Guyer et al. 2022*. D) UMAP plot of the *Guyer et al. 2022* dataset with individual cells colored by module scores generated by the *addmodulescore* function of *Seurat* of the 11.5 dpc wavefront ENCDC (left panel) and 11.5 dpc trailing ENCDC (right panel) gene signature. E) Violin plots with median values of module scores for wavefront ENCDC genes (left panel) and trailing ENCDC genes (right panel) in the *Guyer et al. 2022* dataset. F) UMAP showing subset of developmental pseudotime estimation of cell maturation in *Guyer et al. 2022* neurosphere dataset. G-H) Heatmap representations of significant changes in gene expression over developmental pseudotime for (G) wavefront ENCDC associated genes declining over pseudotime maturation and (H) trailing ENCDC associated genes increasing over pseudotime maturation in neurospheres.

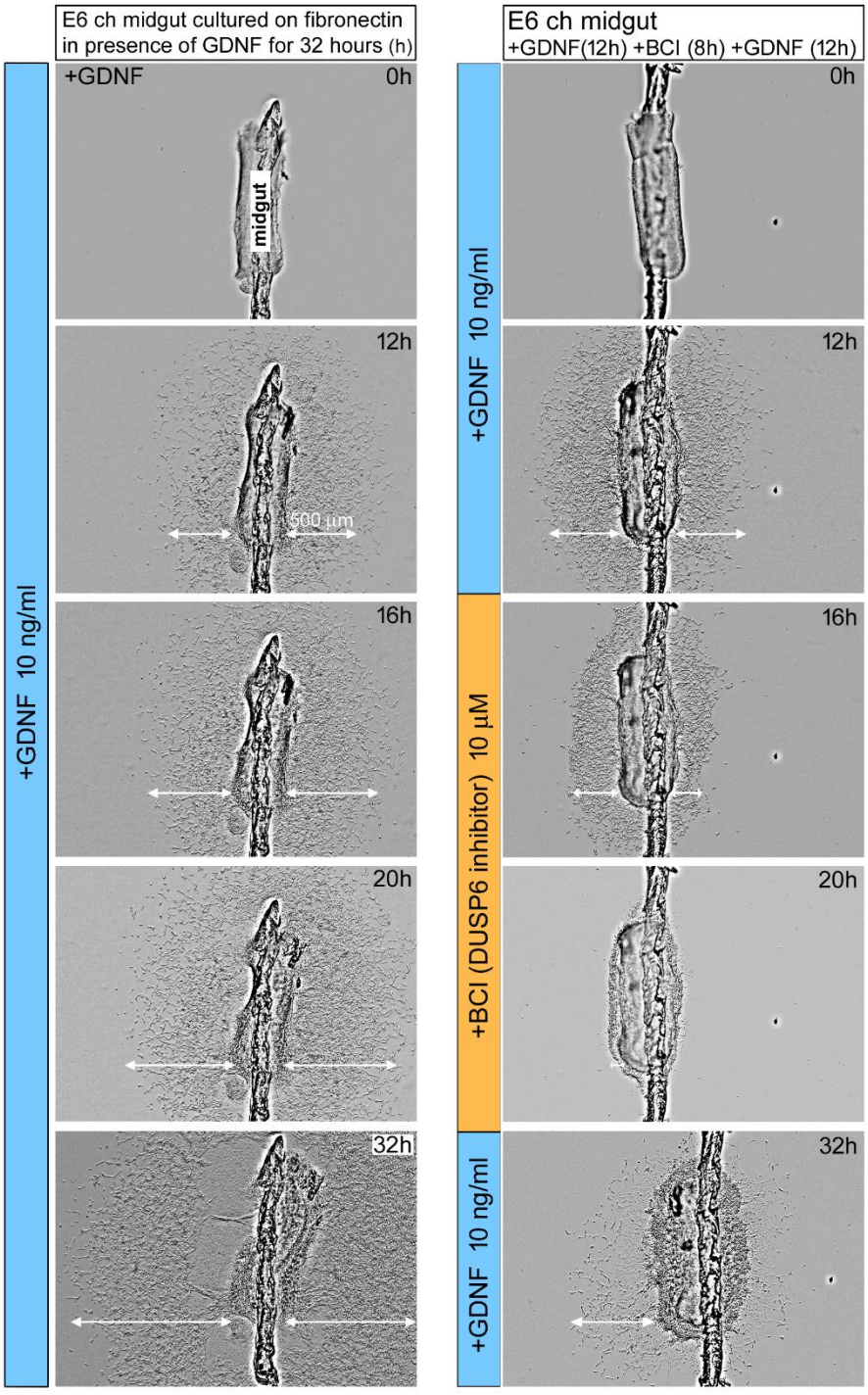

Fig. S3. Migration assay in the embryonic chick midgut. For ENCDC migration assays, E6 (HH29) chick postumbilical midgut was cultured on fibronectin-coated plastic surface in presence of GDNF (10 ng/ml) for 32 hours. Cultures were repeated with removal of GDNF after the first 12 h, followed by 8 h in presence of DUSP6 inhibitor BCI and incubation for another 12 h in presence of GDNF. The addition of GDNF induces a robust ENCDC migration out of the gut before and after application of DUSP6 inhibitor. Top 2 images: GDNF induces ENCDCs to migrate out of the gut, whereas addition of BCI inhibits this effect (ENCDCs return to the explants). Bottom: 8 h later, the addition of GDNF induce again ENCDC migration from the explant.

Table S1. Differential gene expression analysis table.

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Table S2. Gene expression profile of wavefront ENCDCs.

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Table S3. Gene expression of the DEGs in the neuronal and glial trajectory.

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Table S4. Developmental pseudotime analysis in the ENCDCs of the neuron and glia developmental trajectories.

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Table S5. GSEA with gene sets of enteric neuron and glia signatures.

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Table S6. GSEA with gene sets of postnatal enteric nerurospheres.

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Table S7. Data from differential expression analysis between cell clusters.

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Table S8. The expression of wavefront ENCDC-associated genes that were differentially expressed in ENCDC progenitors in both the *Morarach et al 2021* fetal dataset and *Guyer et al 2023* postnatal neurosphere dataset.

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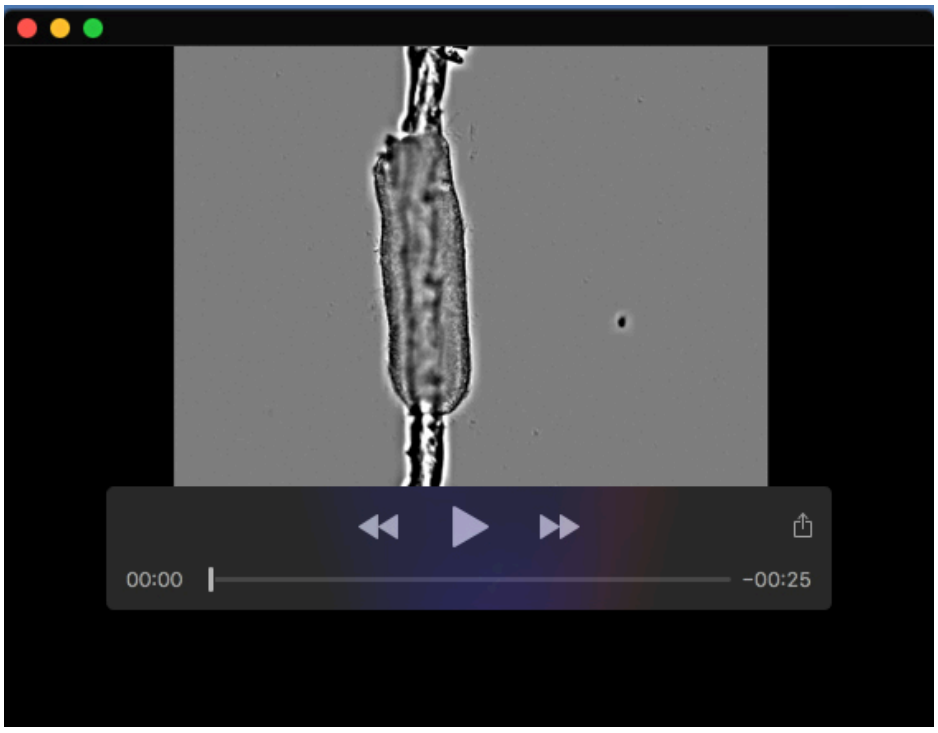
Movie 1. Migration of ENCDCs from E6 chick midgut. The addition of 10 ng/ml GDNF produces a dramatic stimulation of ENCDC migration out of the gut following 32 h in culture.



Movie 2. Live cell imaging recordings of E6 chick midgut explants show that ENCDCs migrating out of gut explants in response to 10 ng/ml GDNF cultured for 12 hours (h), return to the explants after DUSP6 is inhibited with BCI for 8 h. Cells proceed to migrate out of the explants again after BCI is removed. Duration: 32 h.