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Supplementary Information for

The gastrointestinal development 'parts list': transcript profiling of embryonic gut development in wildtype and *Ret*-deficient mice

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Supplementary methods

Mouse strains

The following mouse strains were used in this study:

a) *Ret*$^{CFP/+}$ mice

These mice have been described previously (main text REF 42), but briefly, the floxed *Ret* locus (*Ret*^{fl}) was generated by inserting a gene cassette, comprising the floxed human *RET*9 cDNA with the SV40 intron polyA sequence followed by a cyan fluorescent protein (CFP) cDNA with polyA sequence, into exon 1 of the mouse *Ret* gene; this was functionally equal to the wild-type allele (main text REF 42). *Ret CFP* knock-in mice (*Ret*$^{CFP/+}$) were generated by crossing *Ret*$^{fl/+}$ mice to β-actin-Cre mice to remove the *RET9* cDNA.

b) Myh11transgenic mice

These mice have been described previously (main text REF 39),but briefly a 16kb promoter sequence of the *Myh11* gene was used to drive *Cre* recombinase and EGFP expression. An internal ribosome entry site (IRES) of the encephalomyocarditis virus was added upstream of the EGFP cDNA to permit translation of both *Cre* and EGFP open reading frames from one mRNA. Southern blot analysis showed a single integration site of the transgene and expression in smooth muscle tissues of the developing gut (main text REF 44).

Weighted Gene Co-Expression Network Analysis (WGCNA)

To construct correlation matrices between modules of co-expressed genes and various variables ,we used the “bi-weight mid-correlation” correction method, instead of the Pearson correlation coefficient, because it has improved performance when outliers are present (main text REF 20). We set the parameter pamRespectsDendro to TRUE, and, to obtain smaller (fewer genes per module), more numerous modules. We set the minimum module size to 25 and the deepSplit parameter value to 2. We tested association of each module eigengene, which is the first principal component and represents the module’s expression profile, with each of five binary variables using linear regression: genotype (Ret+/+, RetCFP/CFP); sex (males vs. females); E10.5 vs. non-E10.5; E12.5 vs. non-E12.5; E14.5 vs. non-E14.5. Module associations with p-values ($p<0.00277$) below the Bonferroni-adjusted threshold, accounting for the number of modules,18 in this study, were considered statistically significant.

Taqman qPCR probes

The following Taqman probes and primer sets obtained from Applied Biosystems were used in qPCR as an alternate measurement to detect the expression of genes between 1-5 FPKM in our RNA-seq studies to determine the cutoff for expressed genes:

Syt6 (Mm00490071_m1), *Trpc6* (Mm01151079_m1), *Mnx1* (Mm00658300_g1), *Tead4* (Mm01189836_m1), *Dnajc12*(Mm00497038_m1), *Plekhb2* (Mm01234649_m1), *Cdh23* (Mm04335689_g1), *Kif5a* (Mm00515265_m1) and *Angpt4* (Mm00507766_m1)

The following Taqman probes and primer sets obtained from Applied Biosystems were used in qPCR to detect presence of specifc smooth muscle markers in E10.5 mouse embryonic gut:

*Myh11 (*Mm00443013_m1*), Myog (*Mm00446194_m1*), Myl1 (*Mm00659043_m1*)* and *Myh3(*Mm01332463_m1*).*

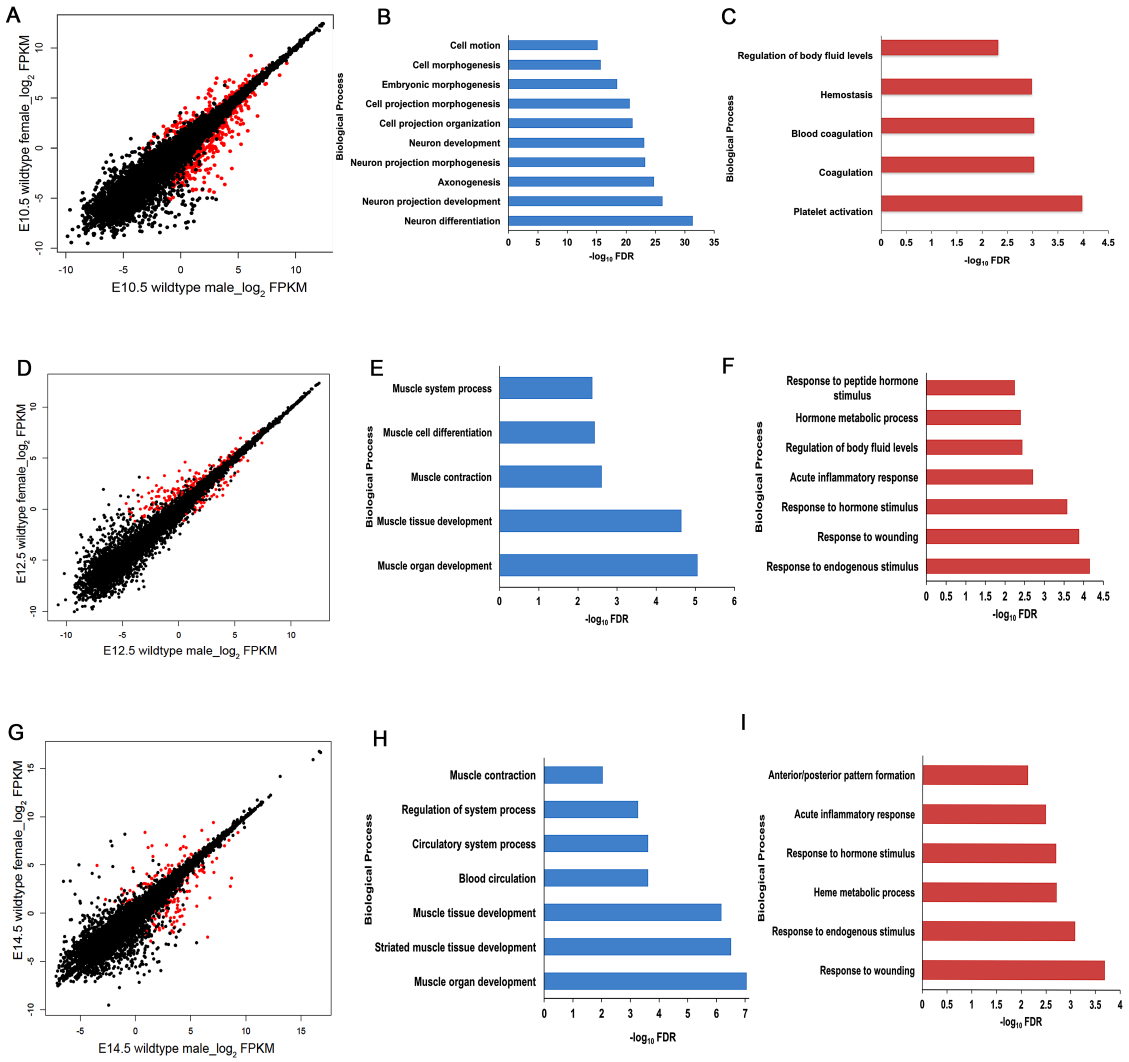

Fig. S1. *Sex-dependent gene expression in the embryonic mouse gut.*
(A) Scatter plot of $\log_2$ FPKM values of genes expressed in wildtype males versus females at E10.5. (B) GO annotation clustering of genes with sex differences shows enrichment of cell motility and neuronal differentiation genes in males, and (C) those controlling homeostasis and blood coagulation processes in females. (D) Analogous analysis of data from E12.5 shows a higher expression of muscle specific genes in males (E) and those controlling hormonal processes and inflammatory responses in females (F). Scatter plot for analogous data at E14.5 also highlights sex-differences in gene expression (G), with higher expression of muscle specification and vasculature genes in males (H) and epithelial morphogenesis in females (I). Genes marked in red in the scatter plots have statistically significant (q-value <0.01) expression differences between the indicated states.

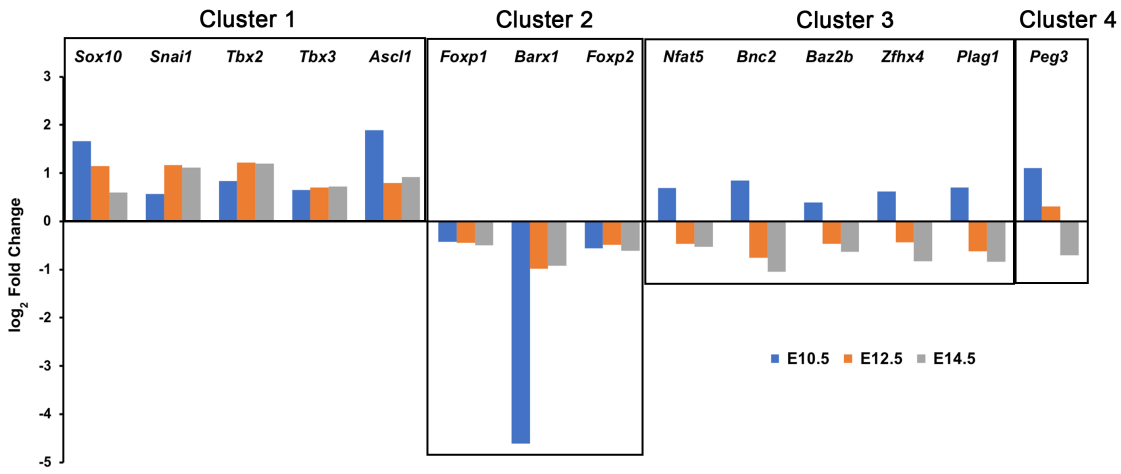

Fig. S2. *Differential effect of Ret loss of function of transcription factors (TFs) through development.*
The 14 transcription factors which are affected in the *Ret* null embryonic guts at all stages in development shows distinct temporal response patterns. Cluster 1 contains TFs which are downregulated all through development whereas cluster 2 contain TFs which are upregulated all through development in Ret null embryos. Cluster 3 are a group of TFs downregulated at E10.5 but upregulated at E12.5 and E14.5 null embryos. Cluster 4 which contain only one TF (Peg3) is downregulated in E10.5 and E12.5 but upregulated at E14.5. The measurement is the log2 scale of the fold change (Wildtype FPKM/ Ret null FPKM) at all 3 stages.

SI Appendix table S1 (separate file)

7793 genes with a mean FPKM ≥ 5 across all 36 samples grouped into 18 modules which were used to test associations between the first principal component (the eigengene) of each module's expression profile with each of five binary variables using linear regression.

SI Appendix table S2 (separate file)

List of genes showing significant differential expression between males and females at E10.5 mouse gut.

SI Appendix table S3 (separate file)

List of genes showing significant differential expression between males and females at E12.5 mouse gut.

SI Appendix table S4 (separate file)

List of genes showing significant differential expression between males and females at E14.5 mouse gut.

SI Appendix table S5 (separate file)

List of genes showing significant differential expression between wildtype male guts at E10.5 (early) compared to E14.5 (late).

SI Appendix table S6 (separate file)

List of genes showing significant differential expression between wildtype and Ret homozygous null mouse guts at E10.5

SI Appendix table S7 (separate file)

List of genes showing significant differential expression between wildtype and Ret homozygous null mouse guts at E12.5

SI Appendix table S8 (separate file)

List of genes showing significant differential expression between wildtype and Ret homozygous null mouse guts at E14.5

SI Appendix table S9 (separate file)

List of transcription factors showing significant differential expression between wildtype and Ret homozygous null mouse guts at E10.5, E12.5 and E14.5