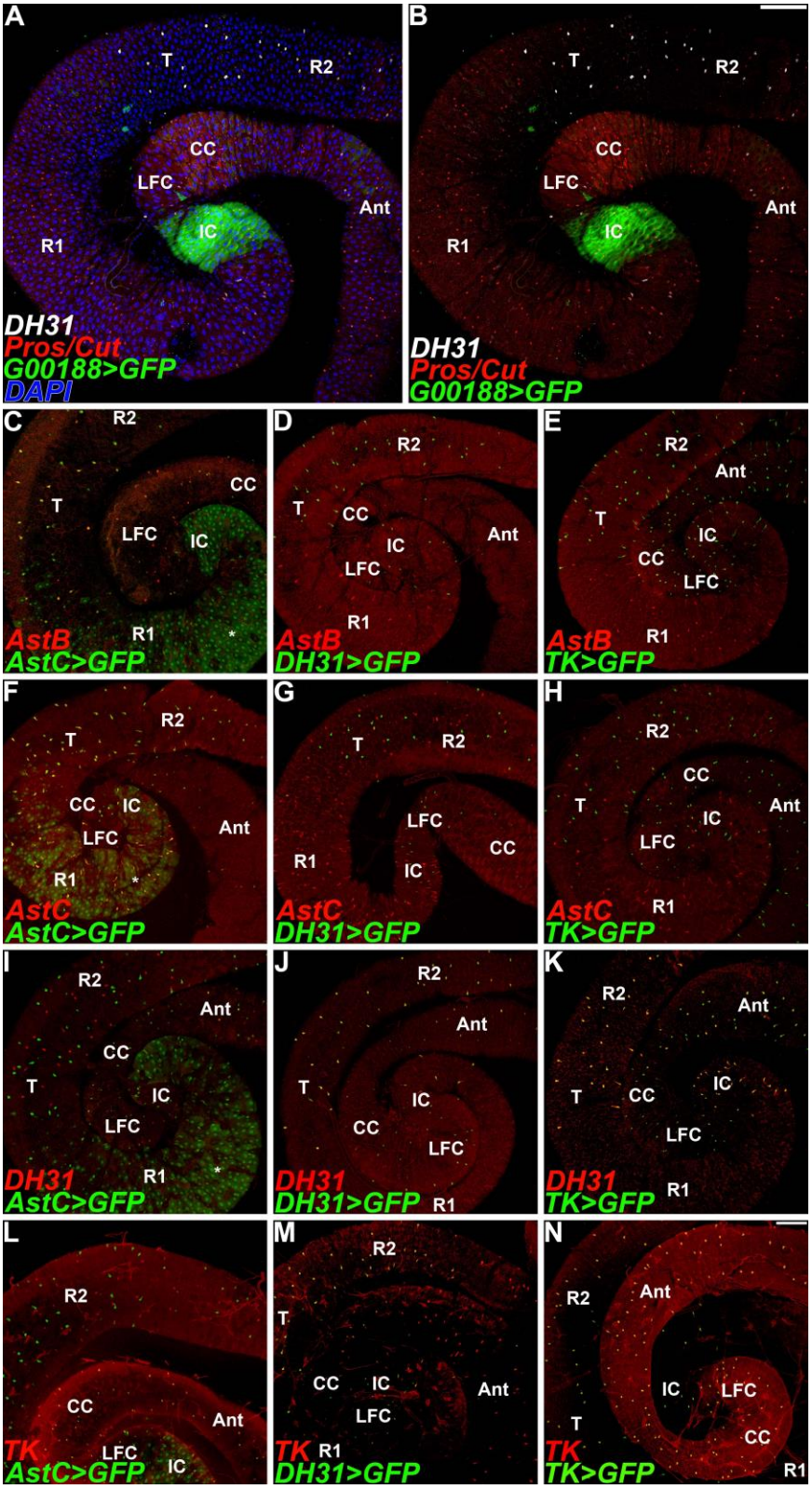
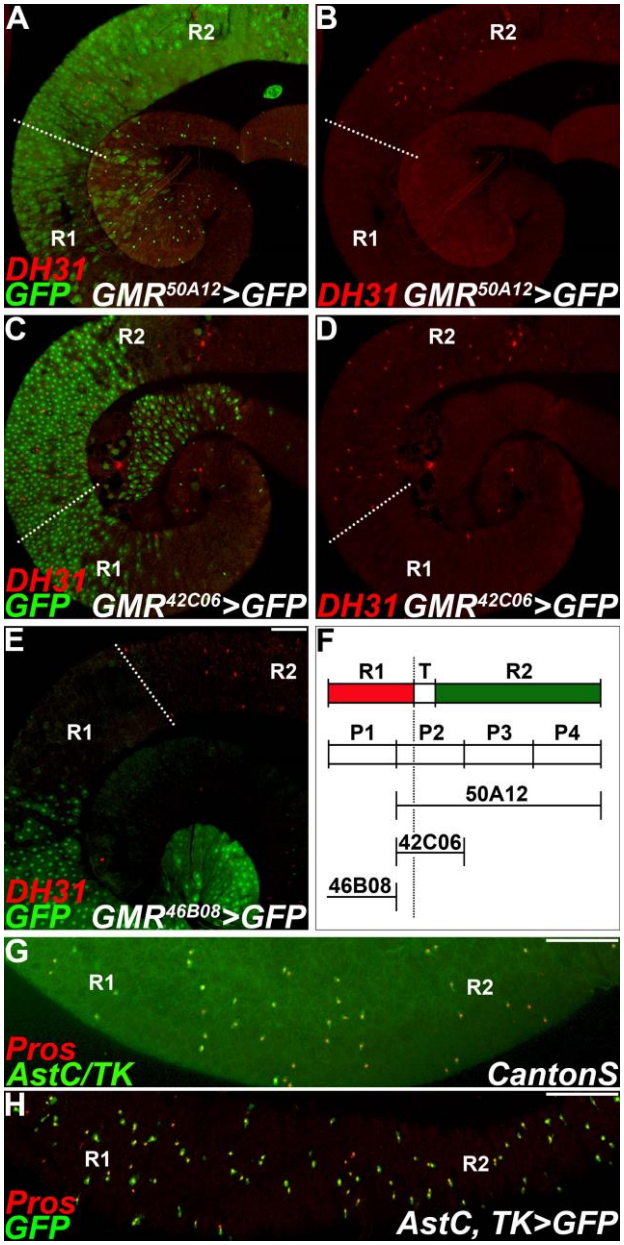
SUPPLEMENTARY FIGURES



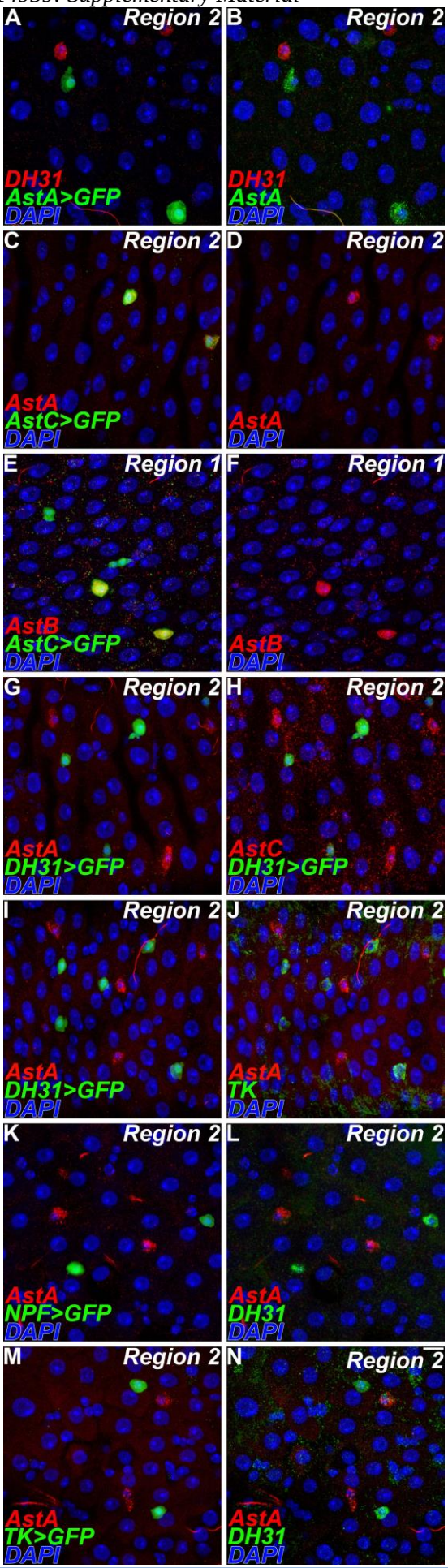
Supplementary Figure 1: Regional differences in adult midgut neuropeptides
(A-N) Morphological and molecular mapping of midgut neuropeptides. Distinct secretory neuropeotides display regional expression along the A/P axis of the adult midgut. (A, B) Anti-DH31, white; anti-Pros/anti-Cut, red; *G00188>GFP*, green; DAPI, blue. (C-N) Neuropeptide antisera, red; neuropeptide *Gal4>GFP*, green.

(C) Anti-AstB, red; *AstC>GFP*, green. (D) Anti-AstB, red; *DH31>GFP*, green. (E) Anti-AstB, red; *Tk>GFP*, green. (F) Anti-AstC, red; *AstC>GFP*, green. (G) Anti-AstC, red; *DH31>GFP*, green. (H) Anti-AstC, red; *Tk>GFP*, green. (I) Anti-DH31, red; *AstC>GFP*, green. (J) Anti-DH31, red; *DH31>GFP*, green. (K) Anti-DH31, red; *Tk>GFP*, green. (L) Anti-Tk, red; *AstC>GFP*, green. (M) Anti-Tk, red; *DH31>GFP*, green. (N) Anti-Tk, red; *Tk>GFP*, green. Asterisk shows non-specific staining in enterocytes associated with *Gal4* line. Anterior midgut (Ant), Copper cell region (CC), Large flat cell region (LFC), Iron cell region (IC), Posterior midgut region 1 (R1), Posterior midgut region 2 (R2). Scale bar: 100 μm.

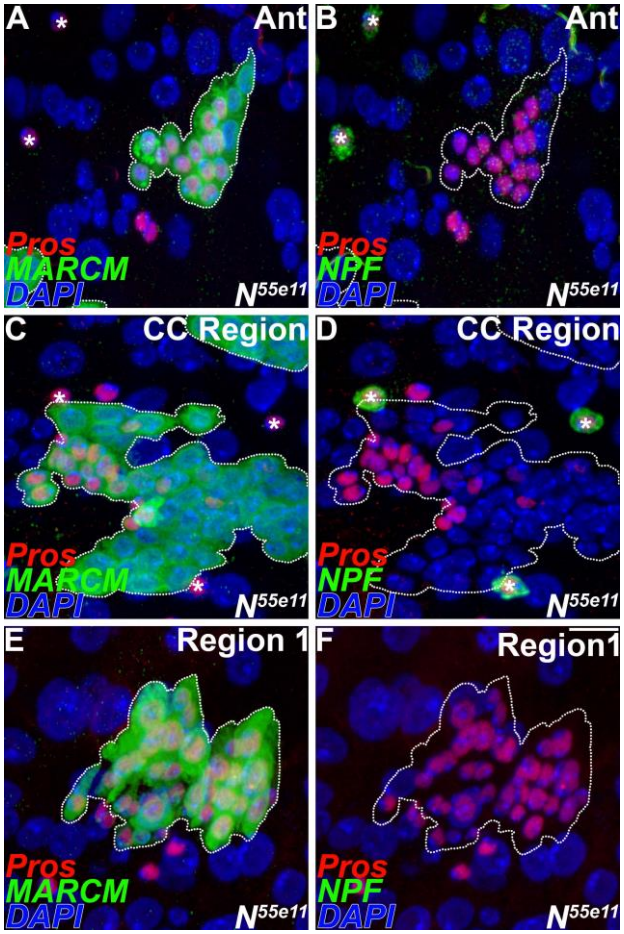


Supplementary Figure 2: Region 1 and region 2 correspond to endogenous domains of neuropeptide expression in the posterior midgut

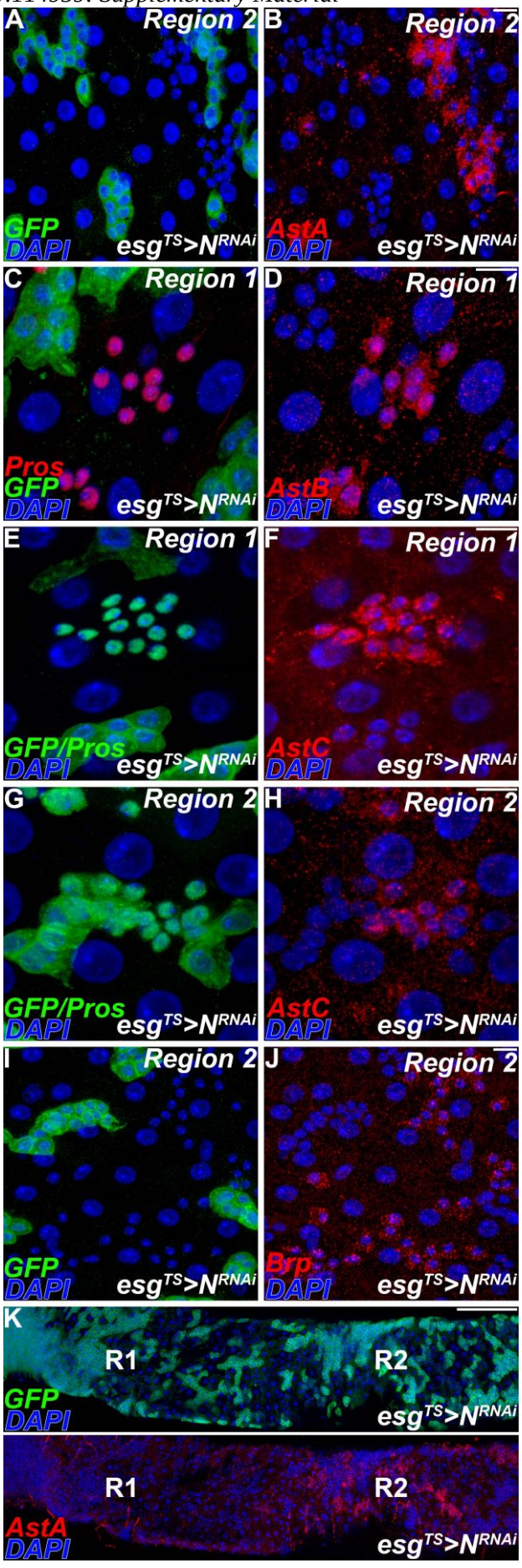
(A-E) A comparison of DH31 protein distribution relative to three previously characterized transgenic *Gal4* marker strains in the adult posterior midgut. (A-E) Anti-DH31, red. The dashed line indicates the boundary defined by the last microscopically verified, brightly stained DH31^{+} cell in the region. (A) *GMR50A12>GFP*, green. (C) *GMR42C06>GFP*, green. (E) *GMR46B08>GFP*, green. (F) Summary diagram depicting boundary alignment of markers examined. Dashed line indicates boundary of DH31 protein relative to three previously characterized transgenic markers. In this study, we employ region 1 (R1) and region 2 (R2) to distinguish regional domains of neuropeptide expression in the posterior midgut. A transitional zone (T) with graded neuropeptide expression is indicated. In our hands, R1, R2 and T boundaries did not strictly correlate with previously characterized markers in the region. Note, the relationship between the relevant nomenclature of Marianes and Spradling, 2013 and Buchon et al., 2013 is as follows: P2=R4c; P3=R5a; P4=R5b. (G, H) Determining the fraction of Pros^{+} cells that label with either class I or class II endocrine markers. (G) Anti-AstC/anti-Tk, green; anti-Pros, red. (H) *AstC>GFP, Tk>GFP*, green; anti-Pros, red. Scale bar: 100 μm.


A
Region 2
DH31
AstA>GFP
DAPI
B
Region 2
DH31
AstA
DAPI
C
Region 2
AstA
AstC>GFP
DAPI
D
Region 2
AstA
DAPI
E
Region 1
AstB
AstC>GFP
DAPI
F
Region 1
AstB
DAPI
G
Region 2
AstA
DH31>GFP
DAPI
H
Region 2
AstC
DH31>GFP
DAPI
I
Region 2
AstA
DH31>GFP
DAPI
J
Region 2
AstA
TK
DAPI
K
Region 2
AstA
NPF>GFP
DAPI
L
Region 2
AstA
DH31
DAPI
M
Region 2
AstA
TK>GFP
DAPI
N
Region 2
AstA
DH31
DAPI

Supplementary Figure 3: Local differences in neuropeptides among adjacent endocrine cells
(A-N) Neuropeptide antisera, red/green; neuropeptide *Gal4>GFP*, green; DAPI, blue. Region 1 (R1), Region 2 (R2). (A, B) *AstA>GFP*. (A) Anti-DH31, red; *AstA>GFP*, green. (B) Anti-DH31, red; anti-AstA, green. (C, D) Anti-AstA, red; *AstC>GFP*, green. (E, F) Anti-AstB, red; *AstC>GFP*, green. (G, H) *DH31>GFP*. (G) Anti-AstA, red; *DH31>GFP*, green. (H) Anti-AstC, red; *DH31>GFP*, green. (I, J) *DH31>GFP*. (I) Anti-AstA, red; *DH31>GFP*, green. (J) Anti-AstA, red; *Tk>GFP*, green. (K, L) *npf>GFP*. (K) Anti-AstA, red; *npf>GFP*, green. (L) Anti-AstA, red; *DH31>GFP*, green. (M, N) *Tk>GFP*. (M) anti-AstA, red; *Tk>GFP*, green. (N) Anti-AstA, red; *DH31>GFP*, green. Scale bar: 10 μm.



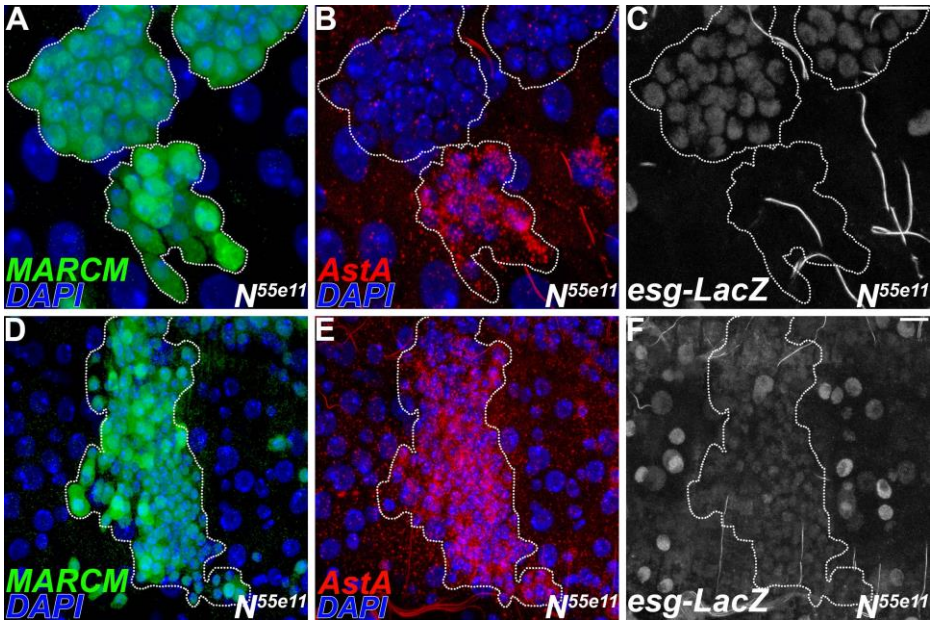
Supplementary Figure 4: Notch is necessary for class II endocrine cells along the length of the adult midgut
(A-F) Mosaic analysis of *Notch* using the MARCM system and a class II enteroendocrine cell marker for the anterior and middle midgut, 5 days after clone induction. Epithelium counterstained with DAPI, blue. Clone outlines indicated with dotted line. Asterisks indicate internal controls. Anti-Pros, red; anti-Npf, green (class II). Note the absence of Npf^{+} cells within the clones. (A, B) Anterior midgut (Ant). (C, D) Copper cell region (CC). (E, F) Posterior midgut, region 1. Negative control. Npf^{+} cells are not present in this region. Scale bar: 10 μm.


A
Region 2
GFP
DAPI
esg^TS>N^RNAi
B
Region 2
AstA
DAPI
esg^TS>N^RNAi
C
Region 1
Pros
GFP
DAPI
esg^TS>N^RNAi
D
Region 1
AstB
DAPI
esg^TS>N^RNAi
E
Region 1
GFP/Pros
DAPI
esg^TS>N^RNAi
F
Region 1
AstC
DAPI
esg^TS>N^RNAi
G
Region 2
GFP/Pros
DAPI
esg^TS>N^RNAi
H
Region 2
AstC
DAPI
esg^TS>N^RNAi
I
Region 2
GFP
DAPI
esg^TS>N^RNAi
J
Region 2
Brp
DAPI
esg^TS>N^RNAi
K
R1
R2
GFP
DAPI
esg^TS>N^RNAi
R1
R2
AstA
DAPI
esg^TS>N^RNAi

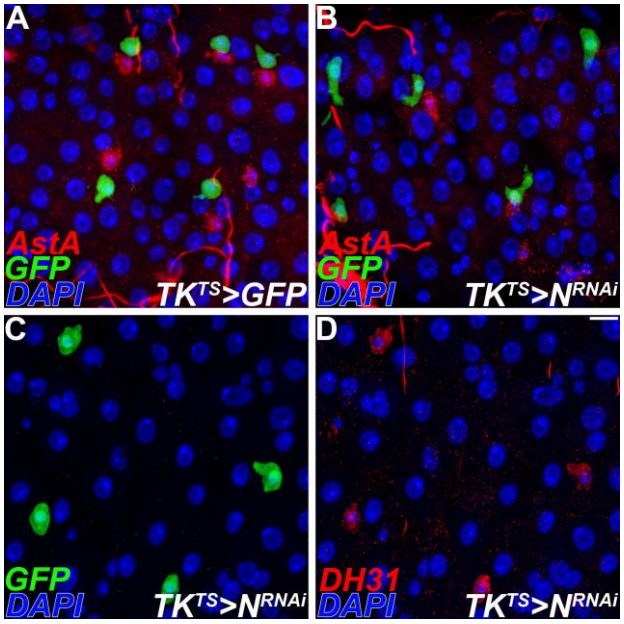
Development 142: doi:10.1242/dev.114959: Supplementary Material

Supplementary Figure 5: Conditional Notch knockdown in midgut progenitors affects local class II endocrine subtype, but not underlying regional identity

(A-K) Distribution of enteroendocrine cell markers following 7 days of conditional *Notch*^{RNAi} knockdown in adult midgut progenitor cells using *esg*^{TS}. Endocrine markers, red; *esg*^{TS}, green; DAPI, blue. (A, B) Anti-AstA, red. (C, D) Anti-Pros/anti-AstB, red. (E, F) Region 1. Anti-AstC, red. (G, H) Region 2. Anti-AstC, red. (I, J) Anti-Brp, red. (K) Anti-AstA, red. Notch knockdown leads to endocrine hyperplasia in region 1 and region 2. Yet, anti-AstA remains localized to region 2 following Notch knockdown. This suggests that Notch controls local, but not regional endocrine cell identity. Scale bars: 10 μm (A-J), 100 μm (K).

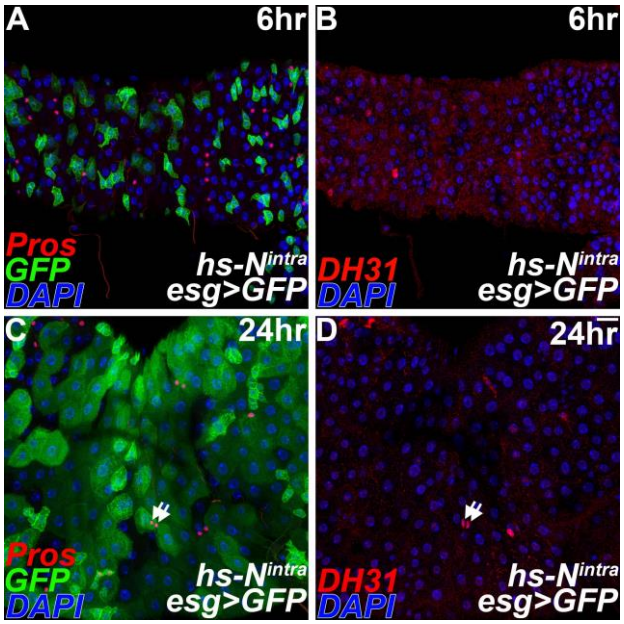


Supplementary Figure 6: Heterogeneity of esg-lacZ in Notch mutant clones
(A-F) Mosaic analysis of *Notch* null clones 10 days following induction using the MARCM system, green; anti-AstA, red; progenitor cells marked with *esg-lacZ*, white. Epithelium counterstained with DAPI, blue. Clone outlines indicated with dashed line. Clones differed in both the levels and extent of *esg-lacZ* present. Scale bar: 10 μm.



Supplementary Figure 7: Notch is dispensable for maintaining class II enteroendocrine cells in the adult midgut

(A-D) Conditional Notch knockdown in *Tk-Gal4*^{TS} cells 7 days following transgene induction. Epithelium counterstained with DAPI, blue. (A) Controls shifted to non-permissive temperature show conditional expression of GPF alone, green. Anti-AstA, red. (B) Notch knockdown with *Tk-Gal4*^{TS} was dispensable for maintenance of anti-AstA in adjacent endocrine cells. (C, D) Notch knockdown with *Tk-Gal4*^{TS} was dispensable for autonomous maintenance of anti-DH31 in endocrine cells. Scale bars: 10 μm.



Supplementary Figure 8: Ectopic class II endocrine cell are produced at low frequency following global induction of Notch^{intra}

(A-D) Induction of *hs-Notch^{intra}* in *esg>GFP* adult midguts. Samples were stained with anti-DH31 and analyzed 6 and 24 hours following heat shock. (A, B) Controls analyzed 6 hr following induction. Stem cell lineages are just beginning to expand. (C, D) 24 hrs following induction, many of the *esg>GFP* progenitors have begun to differentiate as indicated by their increased size. Note the pair of newly produced Pros^{+} cells that are also DH31^{+}, arrows. Scale bar: 20 μm.

Supplementary Table 1: Summary of regional & local neuropeptide expression in the adult midgut

	Ant		CC		LFC		IC		?		R1		T		R2	
Marker	C I	C II	C I	C II	C I	C II	C I	C II	C I	C II	C I	C II	C I	C II	C I	C II
AstA	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
AstA-GFP	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
AstB	-	-	-	-	-	+^	-	+^	-	-	+	-	+	-	-	-
AstC	+^	-	+^	-	+^	-	+^	-	+	-	+	+	+	-	+^	-
AstC-GFP	+	+†	-	+‡	+	-	+	-	+	-	+	+	+	-	+	-
DH31	-	+^	-	+^	-	+^	-	+^	-	+^	-	-	-	+	-	+&
DH31-GFP	-	+*	-	+	-	+	-	+	-	+	-	-	+†	+	-	+
NPF	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-
NPF-GFP	-	+	-	+	-	+	-	+	-	+	-	-	-	+#	-	+#
TK	-	+	-	+	-	+	-	+	-	+	-	-	-	+	-	+
TK-GFP	+†	+	-	+	-	+	-	+	-	+	-	-	-	+	-	+

Anterior Midgut (Ant), Copper Cell Region (CC), Large Flat Cell Region (LFC), Iron Cell Region (IC), Region 1 (R1), Transitional (T), Region 2 (R2), Class I Endocrine (C I), Class II Endocrine (C II).

The following disparities between specific Gal4 lines and their corresponding antibodies are noted:
* Same region; same class, incomplete labeling
† Same region; ~1% labeling of atypical class
‡ Same region; 100% labeling of atypical class
# Atypical region; 100% labeling of same class
^ Low Expression under baseline conditions
& Low Expression in posterior of segment