SUPPLEMENTARY MATERIALS AND METHODS

Drosophila genetics

Driver lines were *Bx-Gal4* (wing disc pouch), *Dpp-Gal4*, and *Ptc-Gal4* (both wing disc antero-posterior boundary). Overexpression lines were *UAS Nicd* (made by the Bray lab), *UAS GFP:Act87E [7-6]* BL#9249, and *UAS Pdp1.T* BL#78087.

Overexpression lines tested in the *Bx-Gal4, UAS GFP;; UAS Nicd, UAS scribHMS01490* screen were *UAS GFP*, *UAS bskK53R [20.1a]* BL#9311, and *UAS SOD CAT* (gift from P. Leopold). RNAi lines used are listed in the table below with an indication of the labels used in Fig. 4E&F. TRiP collection lines have a stock BL#, and Vienna collection lines have a stock v#. While performing the *Bx>NS* modifier experiments, we used controls originating from the same collection: *RNAi white* for TRiP lines, and *RNAi yellow* for Vienna KK lines.

Gene	RNAi ID	Stock #
Act87E	HMS02488	BL#42642
Act87E	KK111781	v#102480
cdi	KK100725	v#109409
CG7059	GD10763	v#21651
CG13850	KK104804	v#100863
CG13857	GD6226	v#44061
CG18596	KK104660	v#108183
ftz-f1	HMS00019	BL#33625
Ir87a	HMJ22848	BL#60476
Ir87a	KK106593	v#100667
kay	HMS00254	BL#33379
p53	HMS02286	BL#41720
Pdp1	HMS02030	BL#40863
Pdp1	KK109014	v#110551
REG	KK102083	v#110156
stat92E	HMS00035	BL#33637

vito	KK111866		v#102513
w	HMS00045		BL#33644
y	KK104196		v#106068
yellow-e	HMC06250		BL#65970
yellow-e	KK106243		v#100926
yellow-e3	KK106158		v#105879
yki	HMS00041		BL#34067

Primers

qPCR primers:

Act5C_F: Act5C_R: GAGCGCGGTTACTCTTTCAC

Act87E_F: ACTTCTCCAACGAGGAGCTG

Act87E_R: Atf3_F: GTCCACCGCAAGTGCTTCTA

Atf3_R: TTTCTTTGGATGGCAGGGCA

Diap1_F: CAGCATGGCAACATTGGGAC

Diap1_R: ATGAAGGCAGTGGCTGAGTC

E2f1_F: CAGCCACACGCATCTTCAAC

E2f1_R: ACTTTGTCACAGAGGAGGCG

Ets21C_F: ACAGAATCCTCGCCTCCAAC

Ets21C_R: ftz-f1_F: GACTGCTGCCGTAGCCTATT

ftz-f1_R: CTGCTCGCTGATTCGTCCAA

mxc_F: TAGGCATACCGCTTTCCGTG

mxc_R: ATTCCTGGTCGGACATGCTT

p38a_F: TTCATGCAGACATAGTCGCCC

p38a_R: ACTAGAGGAGGAGCAGCGAA

CTAGTGGACAGCGGCGTATT

TACGGACAGGTGTCAAAGGC

CAGCGATCCATTAGCGGGAT

p53_F: TGCGTGTGTTCCTTTGCTTC

p53_R: GTTCAGGGGGACTACAACGG

puc_F: ATTGACCTCGCCGCCAATTA

puc_R: ATTCCGCTTGAACAGAGCCA

sd_F: sd_R: AGGGTCCACAGAATGCGTTT

Sdr_F: TCGCTTTCCACCTTCTCCAC

CGCTCCCTCAATCCCAAAGT

Sdr_R: ACAACGTCCATCAGCCAGTT

Ser_F: GCACGAATCTCTGGTGTGGA

Ser_R: TAGATTTGGCTGGCAGTCGG

wg_F: wg_R: GCAGTCTGGTCTGGTCTACG

Wnt10_F: ATTGTGCGGGTTCAGTTGGA

Wnt10_R: AATGGCATCGGTGGAACTGT

CAGCGTCTTGCGATTGATGG

qChIP primers:

E(spl)mβ_F: AAGTCGGAGCTTTGAATGAG

E(spl)mβ_R: CAAGTCATTTTATTGCCCTCAC

E(spl)m5_F: GTTTCCGCAGGTCCAGTTAC

E(spl)m5_R: GTTTGATGTTCACGCTGCTG

white_F: CGAAGGACGTTGACACATTG

white_R: GAATTGCCGCTTTTTCTCAC

DDC_F: AAGTGGGATTTGCCAGTGAC

DDC_R: TGCTGGTGAACTTTGACTGC

CG42808_F: CTCGTTAAGAGCAACTGCGA

CG42808_R: GTGAGAACTCCGAATCGAGG

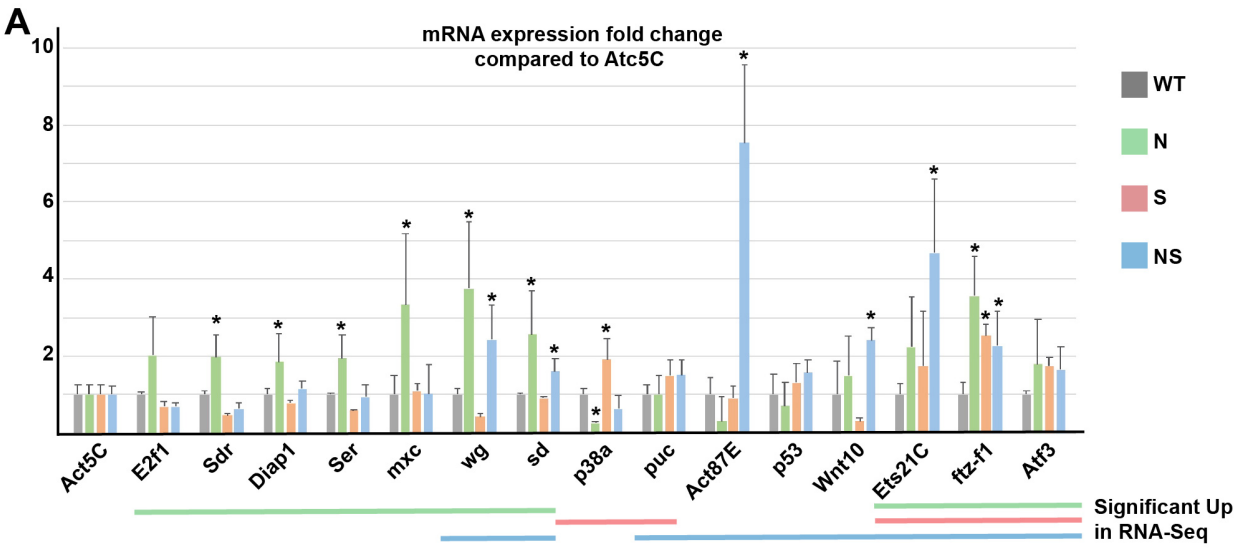
CG6191_F: CGAAAAATGCGGACGATTCC

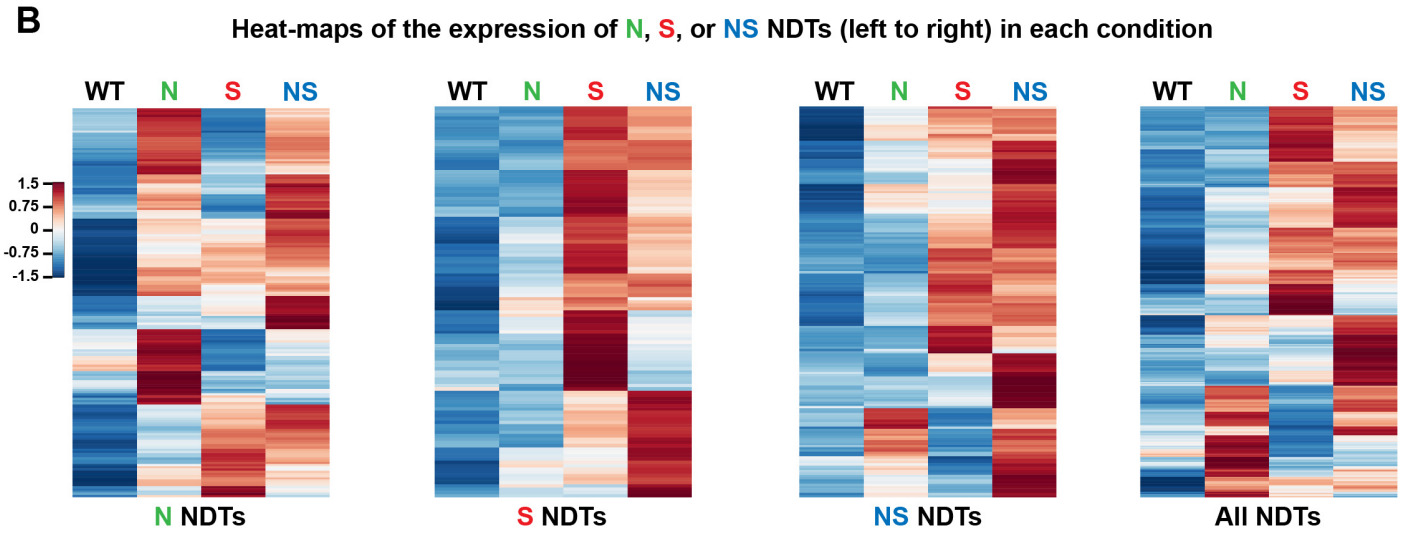
CG6191_R: CCCACCAATCTAGGGTTTCA Ilp8_F:

TCATCTCCGGTGTCTGACTT Ilp8_R:

AAAGAATTGGCTGCGGAAGA

Logeay et al. Figure S1





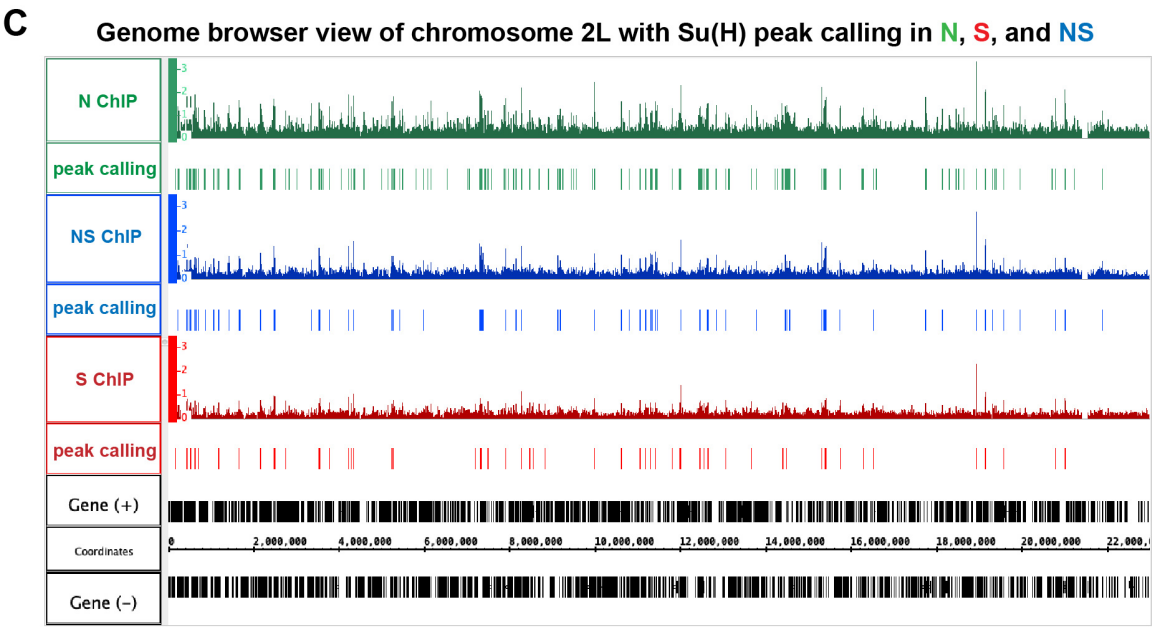


Fig. S1. Features of the Notch Direct Targets (NDTs) in N, S, and NS (relates to Fig. 3)

A. Semi-quantitative RT-PCR of the indicated genes represented as fold change compared to WT (grey) in the different N (green), S (red), and NS (blue) growth paradigms and normalized to *Atc5C* expression. Biological triplicates, standard error to the mean (s.e.m.) is shown. ANOVA statistical test, * $p<0.05$.

B. Heatmaps for the expression of the different NDTs in WT, N, S, and NS. From left to right are presented the N, S, NS, and finally All NDTs, highlighting that NDTs could be transcriptionally up-regulated in more than in one condition.

C. Genome browser view of the whole left arm of the 2nd chromosome, and showing the Su(H) ChIP enrichment (upper rows) and the intervals called as Su(H) peaks (lower rows) in N (green), NS (blue), and S (red). Note the higher number of peaks in N, and the rarity of NS, or S peaks not found in N.

Logeay et al. Figure S2

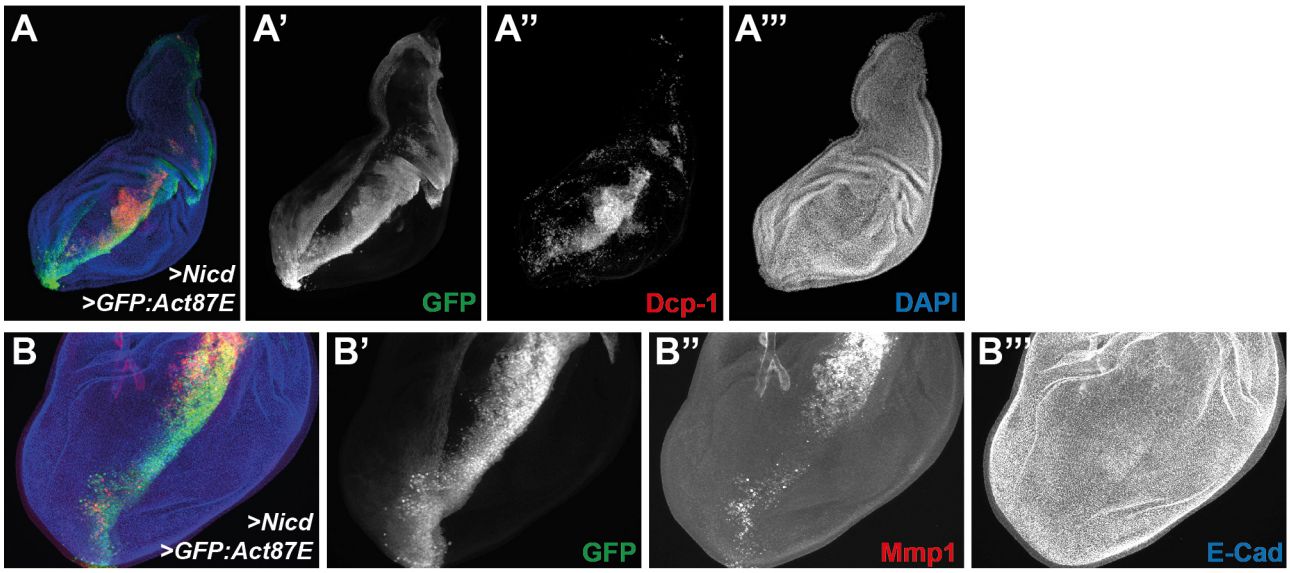


Fig. S2. Act87E promotes Mmp1 expression and cell delamination.

A-B. GFP:Act87E overexpressed together with activated Notch (Nicd) under the control of the *Ptc-Gal4* driver (antero/posterior boundary cells in green, A'&B'), promotes the expression of the Dcp-1 caspase (red, A''), and the metalloprotease Mmp1 (red, B''). Similar results were obtained for the sole overexpression of GFP:Act87E (without Nicd). DAPI (blue, A''') or E-Cad (blue, B''') mark all wing disc cells. (A) Whole wing disc. (B) Detail of the overgrowing wing pouch (magnification in B is twice that in A).

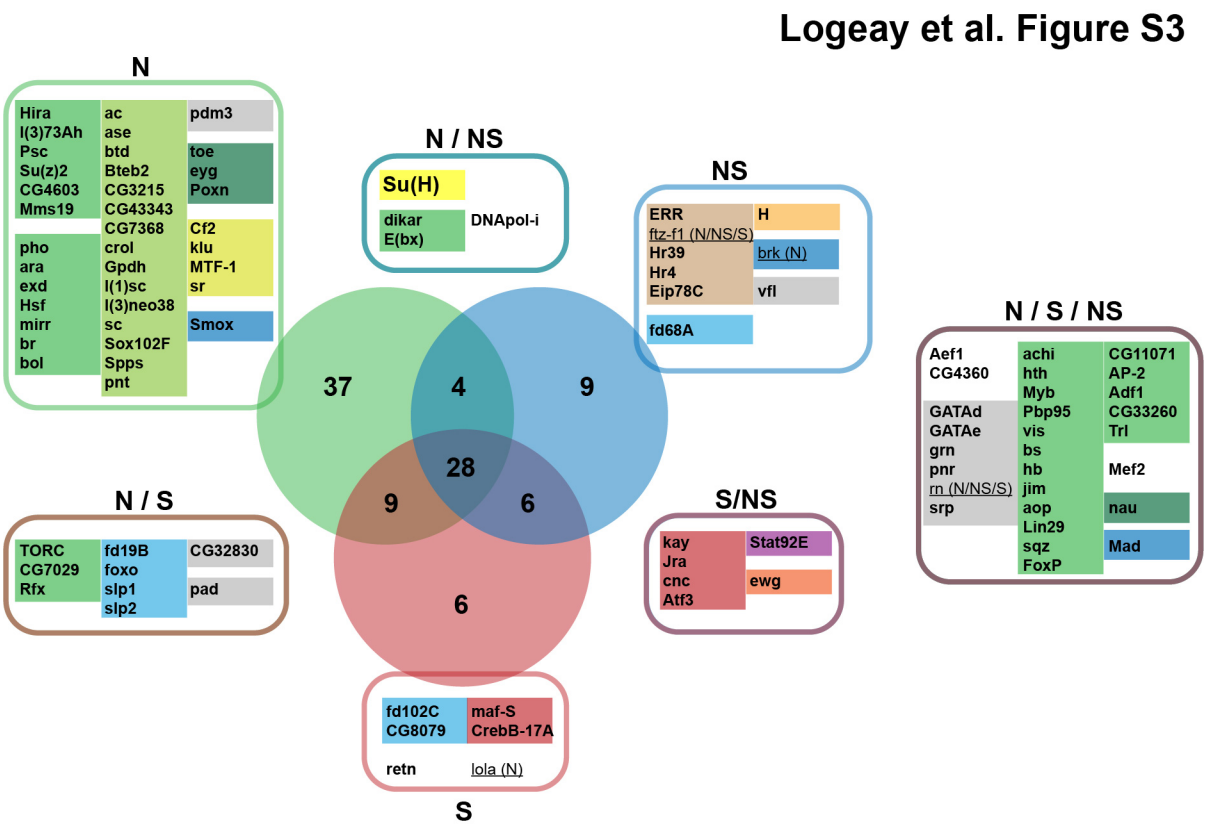


Fig. S3. Identification of potential transcriptional modules mediating N, S, and NS growth (relates to Fig. 3)

Venn diagram of significant transcription factors (TFs) identified by iRegulon as potential key mediators for the expression of the N (in the green circle), S (red circle), and NS (blue circle) Notch Direct Target (NDT) genes. Fed with lists of co-regulated

genes, and analyzing the genomic features in the vicinity of the transcription start sites of these genes, iRegulon identifies potential groups of TFs and DNA-binding factors, that are enriched in the dataset of regulatory sequences, and could thus represent potential mediators regulating the expression of NDT genes in N, S, and NS.

For each condition, TFs were identified as part of "regulons" or group of TFs that could potentially together regulate the expression of subsets of the transcriptome. Taking only the TFs corresponding to the significant regulons identified in N, S, and NS, the shared and unique potentially regulating TFs are here presented in the different colored boxes.

In each box, TFs were then grouped according to their molecular family (suggesting pretty similar binding motifs on the DNA), and color-coded: for instance, bZIP TFs in light maroon, or the nuclear receptors in brown. The molecular family color code was not respected for the green groups which correspond to very diverse "regulons" that appear linked to epigenetic chromatin regulators/remodelers. For the complete list of regulons identified in each condition, and the nature of TFs and DNA binding proteins in each regulon, see Supplemental table S5). Numbers in the Venn diagram represent the number of TFs identified. TFs that are also NDTs and which could participate in feedforward loops are underlined (with their NDT condition in parentheses).

Logeay et al. Figure S4

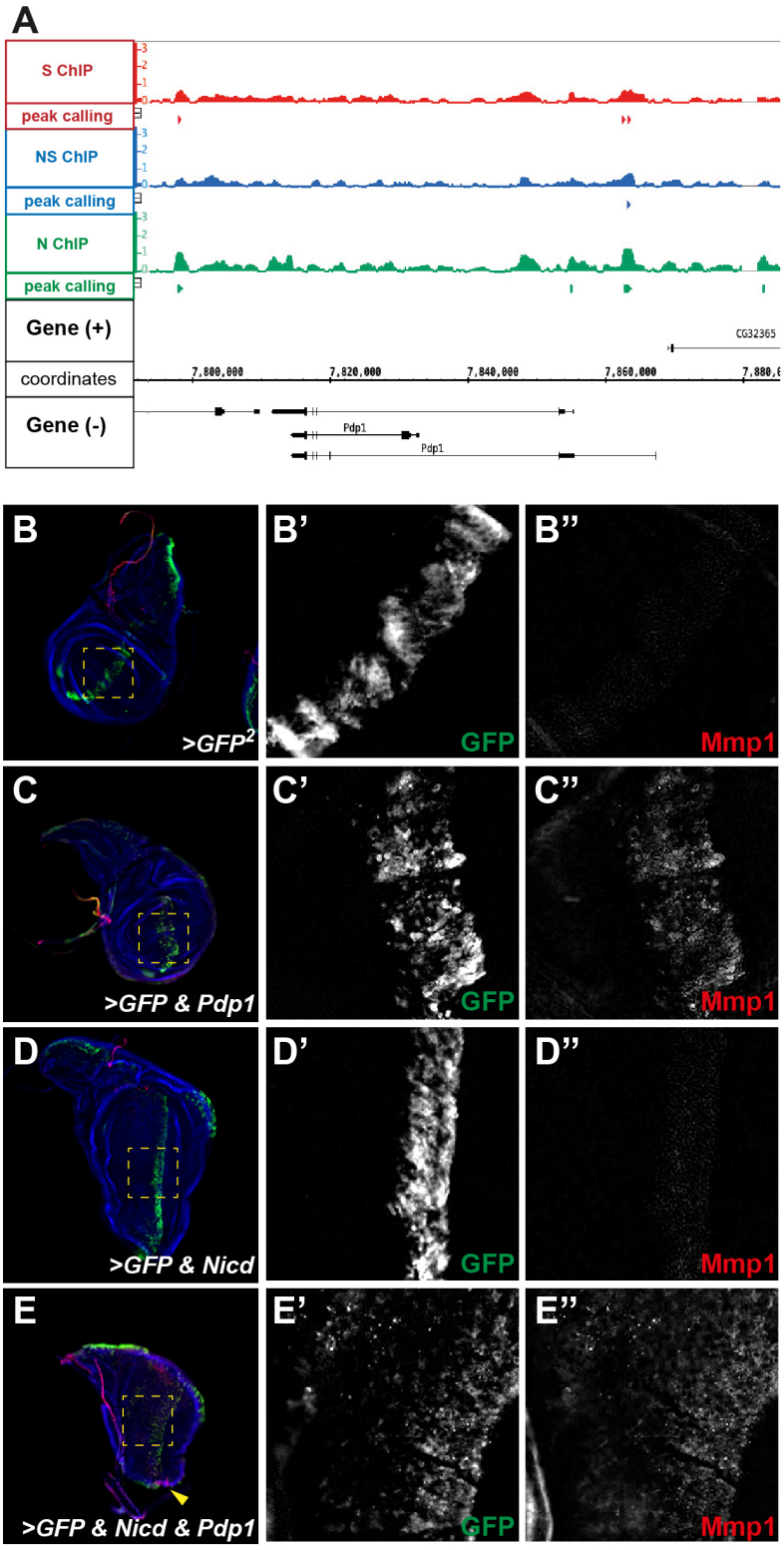


Fig. S4. Pdp1 is a direct Notch target (relates to Fig. 4)

A. Genome browser view of the *Pdp1* locus, and showing the Su(H) ChIP enrichment (upper rows) and the intervals called as Su(H) peaks (lower rows) in N (green), NS (blue), and S (red).

B-E. Pdp1 overexpression causes Mmp1 expression. GFP alone (B) or in combination with *Pdp1* (C), with *Nicd* (D), or with *Nicd & Pdp1* (E) was overexpressed using the *Dpp-Gal4* driver. Higher magnification corresponding to the yellow dashed boxes show GFP in green (B'-E') and Mmp1 in red (B''-E''). E-Cad used as landmark is shown in blue (B-E). Pdp1 overexpression resulted in Mmp1 positive cells extending anteriorly (C). This was enhanced when combined with Nicd (E). Representative discs shown (out of 18 imaged discs from 3 experiments).

(E) Mmp1 expression is found in the *Pdp1&Nicd* overexpressing cells (GFP positive under the influence of the *Dpp-Gal4* driver), but also in non-expressing cells (non- autonomous, yellow arrowhead).

Table S1. Differentially expressed genes in N, S, and NS identified by DESeq (related to Fig. 1).

Columns are:

FBgn_ID: Unique FlyBase gene ID

Symbol: Current FlyBase gene symbol

qval: adjusted p-value for multiple testing

logFC: log2 of the Fold Change “Condition N, S, or NS” / “Control WT”

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Table S2. Su(H) ChIP enrichment peaks coordinates in N, S, and NS (related to Fig. 3).

Columns are:

Exp: N, S, or NS

Chr: Chromosome arm

MIN: smallest peak coordinate

MAX: biggest peak coordinate

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Table S3. All Notch Direct Targets (NDTs) ordered by genomic position. This table includes an indication whether the genes are transcriptionally upregulated or have a Su(H) peak in the vicinity in each N, S, and NS condition. Columns are:

N/NS/S: NDT in the corresponding condition

Type: NDT in different conditions.

FBgn_ID: Unique FlyBase gene ID

SYMBOL: Current FlyBase gene symbol

K_ARM: Chromosome arm location of the gene

MIN (gene pos): smallest gene coordinate

MAX (gene pos): biggest gene coordinate

STRAND: +1 or -1

N Fold: Log2 Fold Change in gene expression N/WT (n.s. not significant)

N ChIP: Su(H) ChIP enrichment peak within 20kb in N (green yes, red no)

NS Fold: Log2 Fold Change in gene expression NS/WT (n.s. not significant)

NS ChIP: Su(H) ChIP enrichment peak within 20kb in NS (green yes, red no)

S Fold: Log2 Fold Change in gene expression S/WT (n.s. not significant)

S ChIP: Su(H) ChIP enrichment peak within 20kb in S (green yes, red no)

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Table S4. Curated iRegulon analyses of the significantly upregulated genes in N, S, and NS (related to Fig. 3). Analyses were performed using the 6K-PWM and 10kb upstream and downstream set-ups.

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Table S5. Curated iRegulon analyses of the Notch Direct Targets in N, S, and NS (related to Fig. S2). Analyses were performed using the 6K-PWM and 10kb upstream and downstream set-ups.

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