1 **Mechanisms underlying the cooperation between loss of epithelial polarity and**
2 **Notch signaling during neoplastic growth in *Drosophila***

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22 **KEY WORDS:**

23 Notch signaling, *Drosophila*, epithelial polarity, neoplasia, cell competition

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25 SUMMARY

26 Aggressive neoplastic growth can be initiated by a limited number of genetic
 27 alterations, such as the well-established cooperation between loss of cell architecture
 28 and hyperactive signaling pathways. However, our understanding of how these
 29 different alterations interact and influence each other remains very incomplete. Using
 30 *Drosophila* paradigms of imaginal wing disc epithelial growth, we have monitored
 31 the changes in Notch pathway activity according to the polarity status of cells and
 32 show that epithelial polarity changes directly impact the transcriptional output of the
 33 Notch pathway. Importantly, we show that this Notch pathway redirection is not
 34 mediated by a redeployment of Su(H), the Notch dedicated transcription factor, but
 35 relies on the cooperation with a combination of oncogenic transcription factors. Our
 36 work highlights in particular the role of the stress response CEBPG homologue
 37 CG6272/Irbp18 and of its partner Xrp1 suggesting that parts of the cellular
 38 competition program might promote neoplastic growth.

39

40 INTRODUCTION

41 In multicellular organisms, cells constantly integrate many stimuli, to adapt their
42 behaviors and responses. How cells achieve this integration is a fundamental question
43 in biology and its disruption underlies many pathologies. Cancer genomes harbor
44 many mutations and understanding how different mutations interact within a given
45 cell is a major goal in cancer research.

46 Epithelial cells represent the basic unit of many organs. Their apico-basal (A/B)
47 polarity is controlled by the asymmetric segregation of highly conserved protein
48 complexes such as the Scrib/Dlg/Lgl complex (Bilder et al., 2003; Coopman and
49 Djiane, 2016; St Johnston and Ahringer, 2010). The far-reaching effects of A/B
50 polarity is epitomized by the observation that many tumors of epithelial origin exhibit
51 impaired polarity, and that several viral oncoproteins target polarity complexes
52 (Banks et al., 2012; Huang and Muthuswamy, 2010).

53 Studies in human cell lines and in animal models have also suggested a contributing
54 role of polarity alterations to tumor formation. For instance, mutations in the baso-
55 lateral determinant *SCRIB1* have been shown to control proliferation and invasion in
56 MCF-10A human mammary cells (Cordenonsi et al., 2011). Similarly in *Drosophila*,
57 *scrib*, *dlg* or *lgl* mutations, result in multilayered overgrowth of larval epithelial
58 imaginal discs (Bilder et al., 2003; Bunker et al., 2015). However, this uncontrolled
59 growth is at least partly achieved because larvae exhibiting *scrib* mutations fail to
60 undergo proper metamorphosis and imaginal discs grow for an extended period.
61 Indeed, *scrib* mutant cells actually grow slower than wild-type cells and are
62 eliminated by wild-type neighbors (Cordero et al., 2010; Igaki et al., 2009, 2006;
63 Ohsawa et al., 2011). Interestingly, this is reversed when additional mutations are

introduced, such as overexpression of the BTB/POZ chromatin remodelers Abrupt or Chinmo (Doggett et al., 2015; Turkel et al., 2013) or the constitutive activation of signaling pathways (e.g. Ras or Notch), converting *scrib* mutant cells into aggressive, invasive and hyperproliferative cells (Brumby and Richardson, 2003; Pagliarini and Xu, 2003). Similar observations have been reported in mouse, where Notch or Ras activation and *Par3* depletion cooperate to generate aggressive neoplasms in mouse mammary glands (McCaffrey et al., 2012; Xue et al., 2013).

The Notch pathway is a highly conserved cell-signaling pathway mis-regulated in several cancers (Ntziachristos et al., 2014; Ranganathan et al., 2011). Upon activation, Notch receptors undergo two proteolytic cleavages to release their intra-cellular domain or NICD, which enters the nucleus, binds to the Notch pathway specific transcription factor CSL (Rbpj in mammals; Suppressor of Hairless, Su(H) in *Drosophila*), and converts it from a repressor to an activator to turn on the transcription of specific target genes (Bray, 2016). These Notch direct target genes differ depending on cell type and account for the variety of outcomes triggered by Notch activity. Increased Notch activity has been associated with several epithelial cancers such as non-small-cell lung carcinomas (Maraver et al., 2012; Ntziachristos et al., 2014), but in animal models, the sole increase in Notch activity either promotes differentiation or only results in benign over-proliferation (hyperplasia) (Brumby and Richardson, 2003; Djiane et al., 2013; Fre et al., 2005; Ho et al., 2015; McCaffrey et al., 2012). However, as mentioned previously, Notch pathway activation cooperates with loss of polarity to generate invasive neoplasms (Brumby and Richardson, 2003; Ho et al., 2015; McCaffrey et al., 2012; Pagliarini and Xu, 2003).

So, while the cooperation between loss of cell architecture and hyperactive signaling pathways is well established, the underlying mechanisms remain poorly understood. It

89 could merely reflect an additive effect where the consequences of both events
90 combine (the additive model). Alternatively, it could indicate a more profound
91 integration within epithelial cells where these two events impact on each other to
92 generate unique new behaviors (the redirective model). Using *Drosophila* paradigms
93 of imaginal wing disc epithelial growth, we have monitored the changes in Notch
94 pathway activity according to the polarity status of cells and show that epithelial
95 polarity changes directly impact the transcriptional output of the Notch pathway. We
96 further provide evidence that this Notch redirection is not mediated by new genomic
97 binding regions for Su(H), but relies on the cooperation with Su(H) of a combination
98 of transcription factors, such as basic leucine zipper (bZIP), whose activity is
99 triggered in response to JNK signaling during polarity loss, extending earlier reports
100 on the cooperation between oncogenic Ras and polarity loss (Atkins et al., 2016;
101 Davie et al., 2015; Külshammer et al., 2015; Uhlirova and Bohmann, 2006). Our work
102 highlights in particular the role of the stress response CEBPG homologue
103 CG6272/Irbp18 and of its partner Xrp1, key factors in mediating the loser fate during
104 cell competition (Baillon et al., 2018; Blanco et al., 2020; Ji et al., 2019; Lee et al.,
105 2018), suggesting that cellular competition, or parts of the cellular competition
106 program, are co-opted during neoplastic growth.

107

108 **RESULTS**

109

110 ***Notch activation and scrib mutation cooperate to promote neoplastic growth***

111 In order to gain insights into the mechanisms underlying neoplastic growth, we first
 112 characterized the effects of Notch activation and *scrib* mutation mediated epithelial
 113 polarity impairment on wing disc growth. Using precisely controlled *Drosophila*
 114 larvae culture conditions (crowding and timing), we compared the phenotypes of
 115 wild-type (WT), *Nicd* overexpressing (N), *scrib* mutant (S), and *Nicd* overexpressing
 116 and *scrib* mutant (NS) 3rd instar wing imaginal discs at 6 days after egg laying at
 117 25°C. These different paradigms are shown in Figure 1A-D. For clarity, in all figures
 118 N will be shown in green, S in red, and NS in blue. Reproducing our previous
 119 observations (Djiane et al., 2013), N discs overgrew compared to WT, but remained
 120 as monolayered epithelia with properly localized E-Cadherin-based adherens
 121 junctions, and represent a paradigm of hyperplastic-like growth (Fig. 1A&B). S discs
 122 were smaller than WT, but grew as unstratified mass of cells with weak uniform E-
 123 Cadherin (E-Cad). These discs however showed an extensive expression of the JNK
 124 signaling target *Mmp1* (Fig. 1C), a metallo-protease implicated in the digestion of the
 125 extracellular matrix, indicative that *scrib*- cells activate JNK signaling and are prone
 126 to invasiveness (Igaki et al., 2006; Uhlirova and Bohmann, 2006). It is noteworthy
 127 that S larvae did not pupariate and if left to grow for longer, the S discs ultimately
 128 developed as massive overgrowths with very disrupted epithelial polarity, that
 129 invaded and fused with neighboring tissues such as other discs (Bilder et al., 2003).
 130 Strikingly, NS discs combined aspects of N and S discs. They were overgrown like N
 131 discs, but also expressed low levels of E-Cad and high levels of *Mmp1* like S discs
 132 (Fig. 1D). These discs grew as multilayered tissues and were able to invade the
 133 surrounding tissues such as haltere discs, and represent therefore a paradigm for
 134 neoplastic-like growth.

135

136 ***Neoplastic and hyperplastic discs have different transcriptomes***

137 The context of the scribble mutation converts the Notch-based *Drosophila* wing disc
 138 into a paradigm of neoplastic growth. Until now, the cooperation between activated
 139 Ras and *scrib* mutations has been studied extensively, mainly in the imaginal eye disc
 140 (Atkins et al., 2016; Cordero et al., 2010; Davie et al., 2015; Igaki et al., 2009;
 141 Katheder et al., 2017; Pagliarini and Xu, 2003; Toggweiler et al., 2016; Wu et al.,

2010), but less attention has been given to the way that other activated pathways, such as Notch or Hedgehog, also cooperate with polarity loss beyond the initial studies (Brumby and Richardson, 2003; Pagliarini and Xu, 2003). Exploring how these other pathways cooperate is important to evaluate the extent that are generally important for neoplasia or specific to Ras signaling induced transformations.

First, we compared the transcriptomes of the different genetic conditions, to identify genes whose expression was significantly different. Differential expression analyses of genome-wide RNA-seq profiles (using DESeq with adjusted p-value for multiple testing <0.05 ; (Anders and Huber, 2010) identified the cohorts of genes that were significantly up-regulated or down-regulated in each condition compared to WT controls (Fig. 1E&F and Supplemental Tables 1-3). The numbers were broadly similar in each condition N (503 up; 663 dw), S (757 up; 1029 dw), and NS (1003 up; 991 dw). Using semi-quantitative qRT-PCR we then validated a subset of the transcriptional changes and could confirm the specific differences. The N only affected genes *E2f1*, *Sdr*, and *mxc*, were activated only in N discs and not in S or NS while the S only gene *p38a* and the NS only genes *Act87E* and *Wnt10*, were activated only in their respective conditions. In addition, *Ets21C*, *ftz-f1*, and *Atf3* were up-regulated in all three conditions, as detected by the RNA-Seq profiles (Fig. 1G). Comparing these data with previously published transcriptome analyses on similar or related genetic backgrounds revealed significant overlap, validating our experimental approaches. For instance, 174 of the 503 up-regulated genes in N, and 285 of the 663 down-regulated genes were also detected in our previous analysis using dual color differential expression arrays (significant overlap $p=1.11e-273$, hypergeometric test, (Djiane et al., 2013). Similarly, 676 of the 757 up-regulated genes in S were identified in a previous analysis of *scrib* depleted discs (significant overlap $p=4.90e-193$; (Bunker et al., 2015).

To gain insights into the nature of the mis-regulated genes we performed a GO term analysis on the genes affected in N, S, and NS ($p\text{-value} < 0.05$). As expected from their genetic composition, the N and NS tissues were over-represented for genes in the Notch signaling pathway (GO:0007219) and the NS and S had changes in A/B polarity (GO:0045197; GO0019991) (Fig. 2A and Supplemental Tables 4&5). Focusing on the other most robust GO terms ($q\text{-value} < 0.05$) revealed common

176 alterations in all three growth paradigms which included cell adhesive properties
177 (GO:0030198 “extracellular matrix organization”; GO:0007155 “cell adhesion”),
178 ribosomal biology (GO:0042274/GO:0042273 “ribosomal small/large subunit
179 biogenesis”) suggesting that protein synthesis might be affected, mis-regulated
180 metabolic processes, in particular with respect to glucose and glycolysis
181 (GO:0006002), or oxidative stress (GO:0006979 “response to oxidative stress”; Fig.
182 2A and Supplemental Tables S4&5).

183

184 Despite these similarities, there were also differences in the profiles. For example,
185 markers of increased proliferation were found in both NS and N, but not S, consistent
186 with the overgrowth phenotypes (e.g. “mitotic cytokinesis” and “mitotic spindle
187 organization”; GO:0000281; GO:0007052). The overgrowing behavior of NS tissues
188 may therefore be driven by the Notch activation (Fig. 2A and Supplemental Tables
189 S4&5) although it should be noted that N was enriched in additional mitosis related
190 categories such as “mitotic metaphase plate congression” or “centriole replication”
191 (GO:0007080; GO:0007099), suggesting a more robust proliferation signature in N
192 tissues. Similarly, both NS and S (but not N) showed characteristics of cell migration
193 (e.g. “border follicle cell migration”; GO:0007298) and cellular stress such as
194 “response to starvation” (GO:0042594), or “response to endoplasmic reticulum
195 stress” (GO:0034976) suggesting that these features in NS were likely contributed by
196 the *scrib* mutation (Fig. 2A and Supplemental Tables S4&5).

197

198 Notably there were several GO categories specific to NS including “positive
199 regulation of apoptotic signaling pathway” (GO:2001235) “negative regulation of
200 SAPK signaling cascade” (GO:0070303) and “mitotic G1/G2 DNA damage
201 checkpoint” (GO:0031571/GO:0007095). These results argue that the combined
202 Notch activation and polarity loss promoted the emergence of new cell behaviors and
203 responses, in particular related to DNA damage responses (Fig. 2A&B and
204 Supplemental Tables S4&5).

205

206 ***Distinguishing between the additive and redirective models of cooperation***

207 The transcriptomic changes revealed that NS encompassed not only additive N and S
208 features but also the emergence of new behaviors. This raises the question of how the
209 defects in *Notch* and in *scrib* cooperate to produce these transcriptional consequences.

210 Two main models could be proposed for genetic cooperation: (1) an additive model,
 211 in which the effect of each genetic change is independent and summed to produce the
 212 phenotype, and (2) a redirective model, in which the two mutations influence each
 213 other to change their effects. Since Notch signaling exerts a direct effect on
 214 transcription, without intermediates (unlike Ras and RTK signaling for instance;
 215 (Bray, 2016), our Notch-based paradigms may allow us to distinguish between the
 216 two models. Indeed, in the N and NS paradigms overexpressing the transcriptional
 217 activator *Nicd*, the genes directly activated by Notch (Notch Direct Targets, NDTs),
 218 should behave differently depending on whether the cooperation is additive or
 219 redirective. In the additive model, genes directly activated by Notch should be
 220 insensitive to the polarity status of the cells and should remain the same in the
 221 hyperplastic (*Nicd* only) and neoplastic (*Nicd* & *scrib*⁻). Alternatively, if the loss of
 222 polarity affects Notch directly, the directly activated genes should be, at least in part,
 223 different.

224
 225 Genes that are directly regulated by Notch (NDTs) should have the transcription
 226 complex, containing Su(H) bound at their regulatory regions (Djiane et al., 2013). To
 227 identify potential NDTs in N and NS, we thus monitored the genomic regions
 228 occupied by the Su(H) transcription factor by genome-wide Chromatin Immuno-
 229 Precipitation (ChIP) (Supplemental Table S6). These overlapped significantly with
 230 our previous analysis of Notch induced overgrowth, suggesting that we have captured
 231 all the robust regions of Su(H) enrichment.

232
 233 Strikingly, there was a strong overlap in the Su(H) bound regions in both conditions:
 234 almost all (416 out of a total of 464) NS Su(H) peaks overlap with peaks present in N
 235 discs. This implies that the vast majority of Su(H) binding remained the same in N
 236 and NS (Fig. 3C & S1C). The overlap was also important between N and S peaks
 237 (447/554 S peaks overlapping with N peaks; Fig. 3C & S1C). These results suggests
 238 that in NS neoplastic discs (and to a lesser extent in S discs), a minority of Su(H)
 239 peaks represent new binding regions compared to hyperplastic N discs, and that the
 240 new NS behaviors are not the consequence of general redistribution of the Notch
 241 specific transcription factor Su(H).

242 We note that the majority of binding in all conditions is associated with intronic
 243 regions (39.70% and 42.89% in N and NS respectively; Fig. S1A), which are

244 frequently the site of enhancers in *Drosophila* patterning and growth genes. There
245 was a subtle shift in the distribution in other regions with a decrease in Su(H) peaks
246 that were associated with promoter regions in NS discs (from 31.55% in N to 23.06%
247 in NS; Fig. S1A) complemented by an increase in Su(H) binding associated with
248 intergenic regions (from 15.51% in N to 20.47% in NS; Fig. S1A), but the
249 significance of this shift is unclear.

250

251 In order to estimate the programs specifically activated by Notch in N, S, and NS, we
252 then intersected the transcriptomic data with the Su(H) ChIP data, considering that
253 upregulated genes located within 20kb of a Su(H) peak were likely NDTs. Using this
254 approach, we identified similar numbers of NDTs in N (177) and NS (171) (Fig.
255 3A&B, Supplemental Table S7). Again, there was substantial overlap with previous
256 data, with 64/177 NDTs in N conditions being identified in our previous study
257 (significant overlap $p=5.89e-96$, hypergeometric test (Djiane et al., 2013). When the
258 N and NS scenarios were compared, 69 genes were common to both and thus
259 represent core NDTs in the wing disc overgrowth. However, a significant proportion
260 of NDTs appeared specific for each condition: 108 for N, 102 for NS (Fig. 3B) which
261 supports the redirective model. Amongst the 102 NS-specific NDTs, only 24 were
262 also NDTs in S, arguing that the difference cannot be explained by specific
263 contribution of the S condition. Taking all of these comparisons into account, there
264 appear to be 78 genes that are NS-specific NDTs. Only a minority were associated
265 with new Su(H) binding regions: around the *87E* locus (*yellow-e3*, *yellow-e*, *Ir87a*,
266 and *Act87E*), and the *94A* locus (*CG18596*, *CG7059*, *CG13857*, and *CG13850*).
267 Amongst these 78 NS specific NDTs are p53, His2Av, and the Tip60 complex
268 component Act87E (Kusch et al., 2004), which have all been linked to DNA damage
269 response and could therefore account for the NS emergent behaviors identified in the
270 GO term analysis (Fig. 2A and Supplemental Tables S4&5). The Venn-diagrams
271 representing the overlaps in N, S, and NS, highlight the extent of additivity (the
272 yellow circles encompassing common NDTs) and the scale that there is redirection of
273 the Notch pathway output (red circles). Both losses and gains occur so that in the
274 conditions where there is neoplastic growth (NS), 78 new NDTs are acquired while
275 108 NDTs are no longer present compared to N (and 70 compared to S). The
276 observation that the NDTs differ substantially between NS and N is in strong support
277 of the redirective model.

278

279 Taken together, our results indicate that Notch activation and *scrib* mediated loss of
280 polarity cooperate both in an additive and redirective manner to promote neoplastic
281 growth, and that the redirection of the Notch pathway transcriptional output is not a
282 consequence of a general redeployment of Su(H) to new active sites. They argue that
283 alterations in the cell architecture can directly influence the outcome of the Notch
284 signaling pathway and support a model in which different genetic injuries within
285 tumor cells could thus influence each other to sustain neoplastic growth.

286

287

288 ***Identification of the transcriptional networks in our different growth paradigms***

289 Using the NDT datasets, we sought to identify the factors that are required for the
290 transition from N hyperplastic to NS neoplastic growth. As the NS transcriptome
291 showed signatures consistent with “response to oxidative stress” (GO:0006979),
292 “cellular response to gamma radiation” (GO:0071480), and “DNA damage
293 checkpoints” (GO:0031571/0007095), we first asked whether interfering with such
294 pathways could block the growth and invasiveness of NS tissues.

295

296 To perform these genetic tests, we generated a stable fly line which overexpressed
297 *Nicd* and *scribRNAi* together with a *GFP* marker under the *BxGal4* driver (driving
298 expression in the pouch of the larval wing discs; *Bx>NS*), and monitored the size of
299 the overgrowth (GFP positive tissue), and its invasiveness potential (Mmp1
300 expressing cells; Fig. 5A). Blocking the oxidative stress response by overexpressing
301 the Reactive Oxygen Species (ROS) sponge CAT and SOD, or knocking down by
302 RNAi the expression of *ATM* or *ATR* (*tefu* or *mei-P26* in the fly), the two master
303 kinases mediating the early response to DNA damage (double or single strand breaks
304 respectively) did not have any significant effect on the NS overgrowth or the
305 expression of Mmp1 (Fig. 5I). Similarly, expression of RNAi or dominant negative
306 forms of the acute stress response and severe DNA damage major effector and NS
307 specific NDT *p53* could not modify the NS overgrowth phenotype (Fig. 5I). While we
308 cannot exclude that the tools used here were not strong enough, these results suggest
309 that even though activated in NS tissues, oxidative stress and DNA damage responses
310 were either not required to sustain NS growth, or that they could compensate for each

311 other converging ultimately on an as yet unidentified core response promoting NS
312 growth.
313 Next we sought to identify transcriptional factors that could account for the
314 cooperation between Notch and polarity loss. We used the iRegulon software to
315 identify the transcriptional network involved. When utilized to analyze genes
316 involved in sustaining the tumorous growth of *RasV12/scrib*- 3rd instar larval eye,
317 wing and leg discs, it highlighted the role of the Hippo pathway terminal effectors
318 Yki/Sd and their targets Myc, Crp, and Ftz-F1, the JNK pathway regulated AP-1
319 factors (in particular Atf3, Kay, and CEBPG), and the Jak/Stat pathway (Atkins et al.,
320 2016; Davie et al., 2015; Külshammer et al., 2015).
321
322 Implementing iRegulon on our step-wise Notch-based paradigms allowed us (i) to
323 identify transcriptional modules unique or shared between polarity loss only (S),
324 proliferation only (N) and proliferation plus invasiveness (NS), and (ii) to assess the
325 conservation of the “oncogenic” modules identified previously with Ras in a Notch-
326 driven neoplastic paradigm. We performed these analyses feeding iRegulon either
327 with the lists of up-regulated genes in N, S, and NS (Fig. 4A & Supplemental Tables
328 S7-11) or with the lists of NDTs (Fig. S2 & Supplemental Table S12-15). We decided
329 to focus our analyses on the up-regulated genes here since: i) the overexpressed *Nicd*
330 triggers transcriptional activation (Bray, 2016) and down-regulated genes might thus
331 represent very indirect effects of the cooperation, and ii) the *Su(H)* module should be
332 found enriched in the regulatory regions of the up-regulated genes and represent thus
333 an internal control to evaluate the bio-informatic predictions. These analyses
334 identified “modules of transcription factors” likely co-regulating the genes identified
335 in N, S, or NS. Modules identified are presented as Venn diagram (Fig. 4A & S4) to
336 highlight common and specific programs in the different conditions. Feeding either
337 up-regulated genes (Fig. 4A) or NDTs (Fig. S2) identified similar modules indicating
338 that the Notch pathway redirection is mediated, at least in part, by transcription
339 factors that broadly affect the whole transcriptome. This is likely also true for other
340 neoplastic paradigms such as Ras, even though this remains to be established.
341 Importantly, iRegulon identified the Notch pathway dedicated transcription factor
342 *Su(H)* in the N and NS transcriptomes.
343

344 First, focusing on NS, which most resembles the *RasV12/scrib*- paradigms, our
 345 analysis identified the same major nodes and oncogenic module as described
 346 previously:
 347 - AP-1 basic Leucine Zipper factors related to stress kinase signaling; *Mmp1* is a
 348 canonical JNK targets and is highly expressed in NS growing discs (Fig. 1D)
 349 - Stat92E of the Jak/Stat pathway; *upd3* coding for the upstream ligand of the Jak/Stat
 350 pathway is indeed highly expressed in NS discs
 351 - Ftz-F1 nuclear receptor
 352 - basic Helix-Loop-Helix factors of the Myc family.
 353 In NS transcriptome, we also identified a contribution of the E(spl) bHLH
 354 transcriptional repressors. E(spl)-HLH genes are canonical Notch targets, and they are
 355 robustly up-regulated in N and NS, in particular E(spl)my-HLH. In NS there were
 356 however two noteworthy differences when focusing only on NDTs, rather than all
 357 differentially transcribed genes. (1) The absence of the E(spl) factors, suggesting that
 358 when considering the whole NS transcriptomes a significant of genes could be
 359 associated with E(spl)-mediated regulation while this is no longer the case when
 360 considering the more restricted number of NS NDTs. (2) The identification of *Ewg*,
 361 the fly NRF1 homologue. In mammals, NRF1 is implicated in the regulation of key
 362 metabolic and respiratory genes (Herzig et al., 2000).
 363
 364 The iRegulon analyses also suggested that the AP-1 bZIP, and the Stat92E signatures
 365 in NS are contributed by S, since they are also detected in the S transcriptomes, while
 366 the Su(H) signature is contributed by N. Interestingly, the bHLH Myc signature is
 367 found in N and S as well as NS. Finally, iRegulon identified a signature for the
 368 Polycomb chromatin silencers specifically in N. Such factors include Pho, a zinc
 369 finger protein which binds to Polycomb Responsive Elements (PREs) and recruits
 370 Polycomb complexes, and the three Polycomb Repressor Complex 1 components Psc,
 371 Su(z)2, and l(3)73Ah. Recently, PRC1 has been associated with specific and
 372 unexpected transcriptional activation at larval stages, raising the possibility that in N,
 373 such genes, normally repressed at embryonic stages, become active (Loubiere et al.,
 374 2020).
 375
 376 ***A polarity-loss oncogenic module***

In order to validate the functional relevance of the transcription factors identified in the Notch-driven neoplastic growth, we then asked whether their depletion by RNAi could alter the growth and invasiveness of the NS tissue, using the *Bx>NS* fly line described previously. We first focused our analysis on the the oncogenic module.

We confirmed earlier reports that blocking JNK activity by the overexpression of a JNK dominant negative construct, strongly abolishes NS driven growth (GFP positive tissue size; Fig. 5B&J) and invasiveness (Mmp1 expression; Fig. 5B&J'). As shown in the *RasV12/scrib-* paradigms, RNAi mediated knock-down of the Jak/Stat pathway terminal transcription factor *stat92E*, the JNK pathway component *Ets21C*, and to a lesser extent *ftz-fl* (Atkins et al., 2016; Davie et al., 2015; Külshammer et al., 2015; Toggweiler et al., 2016) strongly suppressed both growth (GFP) and invasiveness (Mmp1; Fig. 5J&J'). Unlike the *RasV12* models, we did not identify any particular enrichment for Sd/TEAD, the transcriptional factor mediating the effect of Yki and of the Hippo pathway mediated growth in wing discs. However, impairing Yki activity (through RNAi-mediated knock-down) strongly suppressed NS neoplastic behaviors (Fig. 5C&J).

Taken together these results suggest that independently of the oncogenic driver, Ras or Notch, relatively similar tumorous transcriptional networks (AP-1/Yki/Stat/Ftz-fl) are put in place during their cooperation with polarity loss (Atkins et al., 2016; Davie et al., 2015; Külshammer et al., 2015). Given that these nodes were also identified in the S transcriptome, we suggest that they might represent a polarity loss module cooperating with oncogenic signaling pathways Ras or Notch (and likely other pathways such as Hh as was initially reported; (Brumby and Richardson, 2003).

The CG6272/Xrp1 module is required for neoplastic growth

A striking feature of the iRegulon analyses was that the JNK module, which contained classic basic leucine zipper (bZIP) transcription factors such as Jun or Fos, was found in NS and S. As the bZIP factors identified belonged to sub-families involved in different cellular responses including oxidative stress (Maf-S, Cnc, (Sykietis and Bohmann, 2008), polarity loss (Atf3, (Donohoe et al., 2018), ER stress (Atf6, Crc, (Harding et al., 2003; Ye et al., 2000), DNA damage... they were potential candidates to mediate the transcriptional switch. First, we analyzed whether these

different bZIP factors were specifically up-regulated in NS (Fig. 4B). Most factors, with the exception were upregulated in S, suggesting that polarity impairment promotes the expression of many bZIP factors which could alter the response of the transcriptional machinery. A further up-regulation in NS, even compared to S, could be detected for *kay*, *crc*, *maf-S*, *CG7786*, and *CrebB* (Fig. 4B). Strikingly, *cnc* and *Xrpl* were upregulated only in NS (Fig. 4B). It should be noted however, that gene up-regulations do not always indicate functional relevance. Then, in order to identify which bZIP factor/family is most relevant for NS, we therefore systematically depleted by RNAi the different genes highlighted by iRegulon in this bZIP node, irrespective of their potential up-regulations in NS, using the *Bx>NS* fly line.

Amongst the different factors tested, we observed a significant suppression of the invasiveness (*Mmp1*; Fig. 5K') but not of the growth (GFP; Fig. 5K) of NS tissues after impairing the AP-1 factors *kay* and *Jra* (fly homologues of *FOS* and *JUN* respectively), and *CrebB-17A*. Conversely, knocking down the DNA damage response factor *maf-S* led to a dramatic reduction of the GFP outgrowth but without affecting the invasiveness in which the tissue was expressing high levels of *Mmp1* (Fig. 5G&K). It should be noted here that these *maf-S* depleted discs appeared sick with a severely irregular and misshapen GFP domain (Fig. 5G&K, star), suggesting that *maf-S* might be an essential gene, and that the size suppression might merely reflect enhanced cell death. In contrast, we did not observe any significant effect when *cnc*, the fly homologue of *NFE2L1* implicated in oxidative stress response and excision repair (Han et al., 2012; Sykietis and Bohmann, 2008), *Atf2*, *gt*, *CG7786*, *vri* (Fig. 5K), were knocked-down. Neither were any consequences on the phenotype with knock-down of *Atf6* and *crc* (the *ATF4* fly homologue), which have been both linked to the unfolded protein response and ER stress (Fig. 5D&K; (Harding et al., 2003; Ye et al., 2000).

The most complete suppression of the NS behaviors (both GFP overgrowth and *Mmp1* invasiveness) was observed after depletion of *Atf3*, *Pdp1* or *CG6272* (Fig. 5E,H,K). *Atf3* has recently been shown to control the expression of genes involved in the maintenance of epithelial polarity, and to be specifically activated in polarity deficient cells and required in the RasV12/*scrib*- overgrowth models (Atkins et al., 2016; Donohoe et al., 2018). *Pdp1* (the homologue of Hepatic Leukemia Factor -

HLF) has previously been linked to mitotic cell cycle and growth (Reddy et al., 2006), and shown in the *RasV12/scirb*- paradigms to have modest effects on invasion, but none on growth. The role of *CG6272* (a.k.a. *Irbp18*, the homologue of *CEBPG*) was also highlighted in the *RasV12/scirb*- paradigms where it was shown to control growth but not invasion (Atkins et al., 2016). In the context of Notch (*Nicd/scirb*-), the role of *Pdp1* and *CG6272* appeared more essential.

bZIP factors have been shown to act as homo or heterodimers. Knocking down the function of *crc/Atf4*, the classic bZIP partner of *CG6272/CEBPG*, did not have any effect on NS tissue behaviors (Fig. 5D,E,K) whereas the knock-down of *Xrp1* very efficiently suppressed the growth and *Mmp1* expression in NS tissues. This suggests that *CG6272/CEBPG* could be acting in combination with its bZIP partner *Xrp1* in this context (Francis et al., 2016; Reinke et al., 2013). Although the cellular processes controlled by *CG6272/Xrp1* remain to be determined, the fact that *Xrp1* is an early p53 target gene (Akdemir et al., 2007; Link et al., 2013), raises the possibility that its functions could be related to DNA/genome integrity maintenance. This would align with the NS specific GO categories “cellular response to gamma radiation” (GO:0071480), and “DNA damage checkpoints” (GO:0031571/0007095). Indeed, *Xrp1* has recently been linked to cell competition and shown to control the expression of a subset of genes activated in response to Ribosomal protein haplo-insufficiency including *Ets21C*, *upd3*, or *ilp8* in developing wing discs (Baillon et al., 2018; Boulan et al., 2019; Ji et al., 2019; Lee et al., 2018). Strikingly, we observed a very strong overlap between the *Xrp1* transcriptional program (Supplemental Table S16) and the genes activated in NS (60 out of the 171 *Xrp1* positively regulated genes are also upregulated in NS; hypergeometric test, $p=4.71 \times 10^{-21}$), while a much more modest overlap could be observed with N or S (overlap of 17 and 33 with p values of 1.12×10^{-3} and 1.56×10^{-8} respectively). These results support the proposal that the *CG6272/Xrp1* module is functionally important in the acquisition of neoplastic growth.

DISCUSSION

In this study, using Notch-driven paradigms of epithelial overgrowth in *Drosophila* wing discs, we describe the molecular mechanisms underlying the cooperation between Notch and polarity loss during neoplasia. We show that epithelial polarity alterations redirect the transcriptional outcome of the Notch signaling pathway. We further show that this redirection occurs mainly on pre-existing Su(H) bound regions rather than new ones. Finally, we show that similarly to what was previously described for Ras signaling (Atkins et al., 2016; Davie et al., 2015), the cooperation between Notch signaling and polarity loss is controlled by a “tumor transcriptional network” centered around the AP-1/Stat/Yki transcription factors, and including the critical bZIP factors Pdp1 and CEPBG (CG6272/Irbp18). But our analysis uncovered a previously unreported role for the cell competition regulator, and Irbp18 binding partner Xrp1 (Baillon et al., 2018; Blanco et al., 2020; Boulan et al., 2019; Ji et al., 2019; Lee et al., 2018), raising the interesting prospect that neoplastic growth could be mediated at least in part, by co-opting cell competition.

While cancer genomes exhibit multiple mutations in cancer cells, their functional interactions remain difficult to monitor and model. This analysis in the genetically controlled *Drosophila* wing disc, supports a model in which the effects of different mutations do not just simply add up, but interact with each other leading to the emergence of new cell behaviors (redirective model). Indeed, even though the combination showed characteristics that could be attributed to either Notch (proliferation and mitosis), or to polarity loss (invasion/cell migration), it also exerted signs of emerging behaviors such as DNA damage response. Neoplastic tissues appear thus to experience many cellular stresses: DNA damage responses, but also ER and unfolded protein response, starvation, or oxidative stresses. However, even though present, these different stresses and in particular oxidative stress and DNA damage are not individually necessary in the context of polarity loss as blocking them or the cellular response they promote (CAT/SOD overexpression, or inhibition of ATM/ATR) could not significantly suppress the NS tumorous behaviors. These observations suggest that the different stress pathways activated during polarity loss might all converge leading ultimately to the activation of a common core response.

507 While *Drosophila* and mouse models have demonstrated that overactive signaling
 508 pathways cooperate with epithelial polarity impairment to generate neoplastic growth
 509 (Brumby and Richardson, 2003; McCaffrey et al., 2012; Pagliarini and Xu, 2003; Xue
 510 et al., 2013), the vast majority of studies seeking to understand the underlying
 511 mechanisms, have focused primarily on the cooperation between activated RasV12
 512 and *scrib* mutants, especially in *Drosophila* (Atkins et al., 2016; Cordero et al., 2010;
 513 Davie et al., 2015; Igaki et al., 2009; Katheder et al., 2017; Pagliarini and Xu, 2003;
 514 Toggweiler et al., 2016; Wu et al., 2010). Importantly, the current study, investigating
 515 the cooperation between Notch and polarity, shows that many observations made for
 516 Ras can be extended to Notch, suggesting that the paradigms used are not a Ras
 517 specificity but might represent a more general tumor growth paradigm.

518
 519 But even though we could highlight the involvement of a core “oncogenic module”
 520 (Atkins et al., 2016; Davie et al., 2015; Külshammer et al., 2015), there are specifics
 521 that are likely oncogene specific. In the case of Ras, it was shown that Yki activity
 522 could reprogram Ras by promoting the expression of the Ras pathway specific
 523 regulators Capicua and Pointed to promote aggressive growth (Pascual et al., 2017).
 524 Both genes were either unaffected (*capicua*) or downregulated (*pointed*) in NS Notch
 525 driven neoplastic paradigm, suggesting that, even though Yki is clearly active (Fig.
 526 5C), changes in the expression of *capicua* and *pointed* are unlikely mediators here.
 527 Furthermore, in NS transcriptome, we identified a contribution of the E(spl) bHLH
 528 transcriptional repressors, canonical Notch targets (Bray, 2016), which represents thus
 529 a Notch specificity. However, the fact that motifs for E(spl)-HLH repressors are
 530 found in the up-regulated transcriptome of NS and not N could suggest that in NS, the
 531 repressive ability of E(spl)-HLH factors is antagonized (even though their expression
 532 is not affected). It would be interesting to explore further the link between NS and
 533 E(spl)-HLH-mediated repression, but due to the high redundancy between the seven
 534 E(spl)-HLH factors (δ , γ , β , 3, 5, 7, 8) and Dpn, the requirement of E(spl)-HLH-
 535 mediated repression in the Notch-driven neoplasia could not be formally tested.

536
 537 Amongst the most dramatic suppressors of the neoplastic growth are the two basic
 538 leucine zipper transcription factors CG6272/Irbp18 (the fly homologue of CEBPG),
 539 and Xrp1. These two genes, and in particular *Xrp1*, were up-regulated in NS

540 compared to WT or N. Importantly, impairing with their function in wild-type tissues
541 did not have any effect, confirming observations made by other groups for CG6272
542 (Atkins et al., 2016), supporting a model in which these genes are dispensable in
543 healthy wild-type cells, but become indispensable for tumor cells, hence representing
544 a possible attack strategy specifically targeting tumor cells, while sparing healthy
545 tissues. In mammals, CEBPG represents a major regulator of stress responses. It is
546 recruited through its interaction with the bZIP factor ATF4 at the level of cis-
547 regulatory C/EBP:ATF response elements to activate the expression of stress
548 mitigation genes such as glutathione biosynthesis pathway genes in the case of
549 oxidative stress (Huggins et al., 2015). Interestingly, we did not observe any effect
550 knocking down *Crc*, the fly *Atf4* homologue, suggesting that during NS overgrowth in
551 *Drosophila*, CG6272/Irbp18 could be acting in combination with another partner.
552 CG6272/Irbp18 also interacts with Xrp1, and the heterodimer has been implicated in
553 DNA repair (Akdemir et al., 2007; Francis et al., 2016). We thus propose that the
554 Irbp18/Xrp1 dimer is activated in NS and is required to facilitate DNA repair and
555 ensure genomic stability to prevent catastrophic genotoxic effect upon the combined
556 cellular stresses of S and replication stress of N. Recently, *Xrp1* together with its
557 binding partner CG6272/Irbp18 (but not Atf4) has been shown to mediate a loser
558 status in ribosomal genes deficient cells, through the implementation of a specific
559 transcriptional program (Baillon et al., 2018; Blanco et al., 2020; Ji et al., 2019; Lee et
560 al., 2018). This “loser state” promoter role of Xrp1/Irbp18 is however in conflict with
561 our observation that *Xrp1* is strongly upregulated in NS overgrowing discs, and that
562 *Xrp1* and *Irbp18* are required for the overgrowth and invasive capacities of NS
563 neoplastic cells. This could be reconciled by proposing that in NS neoplastic discs, the
564 role of the Xrp1/Irbp18 is modified by an as yet unknown factor. Alternatively, and
565 more attractively, it is also possible that in NS cells, cell death is prevented as was
566 shown for RasV12 expressing cells (Pinal et al., 2018), for instance by upregulating
567 DIAP1 (a Notch direct target, (Djiane et al., 2013) leading to a perverted cell
568 competition. There an incomplete “loser” program would be initiated, but not fully
569 implemented (no cell death), leading to the secretion of growth factors (e.g. the
570 Jak/Stat ligands upd...) that would act in an autocrine manner to further promote their
571 growth. These factors do not actually need to act cell autonomously, since it was
572 shown that at least in the *RasV12 / scrib-* paradigm, delaminating cells cooperate with
573 non-delaminating proliferating cells to sustain tumor growth (Muzzopappa et al.,

574 2017; Uhlirova et al., 2005; Wu et al., 2010). In NS, preventing the
575 losing/delaminating cells by impairing the Xrp1/Irbp18 nexus, would thus prevent
576 neoplastic growth. This intriguing possibility highlights that more studies are needed
577 to better understand the role of the Xrp1/Irbp18 module, its links to cell competition,
578 and to the growth of neoplastic tissues.
579

MATERIALS AND METHODS

Drosophila genetics

The different overgrowth paradigms were obtained by generating random clones in 3rd instar wing discs at high frequency as previously published in (Djiane et al., 2013). In brief, the *abxUbxFLPase; Act>y>Gal4, UAS GFP; FRT82B tubGal80* flies were crossed either to *FRT82B* (to generate Ctrl discs), or to *UAS-Nicd; FRT82B* (to generate hyperplastic N discs), or to *UAS-Nicd; FRT82B scrib1* (to generate neoplastic NS discs). *scrib1* represents a loss of function allele for the *scribble* gene. Because *scrib1* clones are eliminated in growing discs, the dysplastic S discs were obtained from *FRT82B scrib1 / Df(3R)BSC752* 3rd instar larvae. All crosses were performed at 25°C and carefully staged (time after egg laying and tube crowding). For functional studies, neoplastic growth was obtained by driving *UAS-Nicd* and the *scrib* RNAi *P{TRiP.HMS01490}attP2* by the *Bx-Gal4* (pouch of larval wing discs). Modifications of the overgrowth phenotype and of the expression of the Mmp1 invasive marker were performed by crossing in F1 *Bx-Gal4, UAS GFP;; UAS Nicd, UAS scribHMS01490* to the desired *UAS RNAi* or control lines (*UAS white RNAi* or *UAS GFP*), to ensure similar UAS load. List of lines tested in Supplemental Materials.

Information on gene models and functions, and on *Drosophila* lines available were obtained from FlyBase (flybase.org – (Thurmond et al., 2019).

RNA extraction and RNA-Seq

RNA from 60 or 80 dissected third instar larva wing discs of WT, N, NS and S discs was extracted using TriZOL. Genomic DNA was eliminated using Ambion's DNA-free kit (#AM1906). cDNA bank preparation were then performed from 1µg of RNA and sequencing on a Illumina HisSeq 2000 by the Biocampus genomic facility MGX of Montpellier. After sequencing, reads obtained were filtered based on their quality (circa 40 millions reads were kept per conditions). The reads were then align on *Drosophila* dm6 genome by the ABIC facility in Montpellier producing a matrix of reads per gene and per condition. This matrix was then normalized and pair-wise differential expression was performed using DESeq (Anders and Huber, 2010). Other differential expression tools were tested such as DESeq2 and edgeR with default parameters but appeared either less stringent, or inadequate.

614 **qPCR**

615 qPCR was performed on biological triplicates on a Roche LightCycler 480, and fold
616 change was estimated by the $\delta\delta$ CT approach. List of primers used in Supplemental
617 Materials.

618

619 ***Su(H) Chromatin Immuno Precipitation***

620 After dissection in PBS 1X, Protein/DNA complexes from 60 wing discs (80 for S
621 condition) were cross-linked with 1% formaldehyde for 10 minutes. The reaction was
622 then quenched by 0.125 M Glycine and washed 3x in PBS. Wing disc cells were
623 resuspended in 50 μ L Nuclear Lysis Buffer (Tris-HCl pH 8.1 20mM, EDTA 10mM,
624 SDS 1%). Lysates were sonicated on a Bioruptor (Diagenode), and diluted 10x in
625 Immunoprecipitation Dilution Buffer (Tris-HCl pH 8.1 20mM, EDTA 2mM, SDS
626 0.01%, NaCl 150mM, Triton X-100 1%) and precleared with rabbit IgG (Sigma) and
627 protein G Agarose (Santa Cruz Biotechnology). ChIP reactions were performed by
628 incubating lysates overnight at 4°C with 1ng of Goat anti-Su(H) (Santa Cruz
629 Biotechnology, sc15813), and immunocomplexes were then isolated with Protein G
630 Agarose for 2h, washed 2x with Wash Buffer 1 (Tris-HCl pH 8.1 20mM, EDTA
631 2mM, SDS 0.1%, NaCl 50mM, Triton X-100 1%) and 2x with Wash Buffer 2 (Tris-
632 HCl pH 8.1 10mM, EDTA 1mM, LiCl 250mM, NP-40 1%, Deoxycholic acid 0.4%),
633 before a decross-linking step at 65°C in 0.25M NaCl. Samples were then treated with
634 0.2 mg/mL proteinase K and 50mg/mL RNase A. The DNA was then purified on
635 columns (Qiagen, 28106). ChIP efficiency was checked by qPCR normalized on input
636 chromatin with the following primer couples, corresponding to known strong binding
637 sites of Su(H). List of primers used in Supplemental Materials.

638 For whole-genome analysis, 1 μ g double-stranded ChIP or input DNA (corresponding
639 to 180 discs for each replicate) was labelled with either Cy3- or Cy5-random primers
640 using the Nimblegen Dual Colour kit. Both ChIP and input were co-hybridised to
641 NimbleGen D. melanogaster ChIP-chip 2.1 M whole-genome tiling arrays in the
642 NimbleGen hybridisation station at 42°C for 16 h and then washed according to the
643 NimbleGen Wash Buffer kit instructions. The data obtained were normalized using
644 quantile normalization across the replicate arrays in R. Window smoothing and peak
645 calling were performed using the Bioconductor package Ringo (Toedling et al., 2007)
646 with a winHalfSize of 300 bp and min.probes = 5. Probe levels were then assigned P-

values based on the normalNull method, corrected for multiple testing using the Hochberg–Benjamini algorithm and then condensed into regions using distCutOff of 200 bp.

In order to determine the Notch Direct Targets (NDTs), ChIP and RNA-Seq results were compared: NDTs are defined as up-regulated genes with Su(H) enrichment within 20kb. As such one Su(H) peak could be assigned to several upregulated genes consistent with its role in enhancer regions. The 20kb window was chosen as it allowed the recovery of more than 85% of NDTs in our previous study that was based on closest gene assignment irrespective of distance (Djiane et al., 2013).

GO Term analyses

The lists of significantly regulated genes in the various comparisons were submitted to gene ontology (GO) term enrichment analysis. We used the GO biological process (GOBP) ontology and applied hypergeometric tests (p-values) followed by Benjamini-Hochberg multiple hypothesis correction (q-values).

iRegulon analyses

In order to determine the likely transcriptional modules in our transcriptomic and NDTs datasets, we used the online tool iRegulon (<http://iregulon.aertslab.org/>), with the standard settings using the 6K Motif collection (6383 PWMs) and a Putative regulatory region of “10kb upstream, full transcript and 10kb downstream”. Importantly, these settings allowed the recovery of the “positive control” Su(H) module.

Immunocytochemistry

Antibody staining of wing imaginal discs were performed using standard protocols. Briefly, larval heads containing the imaginal discs (LH) were dissected in cold PBS and fixed for 20min in 4% Formaldehyde in PBS at room temperature (RT), before being rinsed 3x 10min in PBS 0.2% TritonX100 (PBT), and blocked in PBT + 0.5% BSA (PBTB) for 30min at RT. LH were then incubated overnight at 4°C with primary antibodies in PBTB. LH were then rinsed 3x 10min in PBT at RT and before being incubated with secondary antibody in PBTB for 90min at RT. LH were then rinsed 3x 20min in PBT at RT, before being equilibrated overnight in Citifluor mounting media (Agar). Discs were then further dissected and mounted. Images were acquired on a

681 Zeiss Apotome2 microscope and processed and quantified using Zen or ImageJ.
 682 Primary antibodies used were rat anti-DE-Cadherin (DCAD2, Developmental Studies
 683 Hybridoma Bank – DHSB, 1:25), rabbit anti-GFP (A6455, Molecular Probes, 1:200),
 684 and mouse anti-Mmp1 (3A6B4, DHSB, 1:25). Secondary antibodies used conjugated
 685 to Alexa-350, Alexa-488, or Cy3 were from Jackson Labs Immuno Research (1:200).
 686

687 *Quantification methods*

688 Genotypes were tested in batches with controls and 13-17 images corresponding to
 689 13-17 different discs were all acquired on the same microscope with the same
 690 exposure settings.

691 Growth was estimated by the size of the GFP positive area and normalized to that of
 692 controls. A disc was considered overgrown if its size was over the average size of
 693 controls plus 1.5 the stdev of controls. A disc was undergrown, if its size was under
 694 the average of controls minus 1.5 the stdev of controls. Using this “conservative” set
 695 up identified only circa 5% of controls as undergrown.

696 Mmp1 intensities were ranked as High, Low, Null by independent observer with
 697 genotypes masked and processed in random order.

698

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711

712

713 **AUTHOR CONTRIBUTIONS**

714 RL, CG, PL, DK, and AD performed experiments; RL, CG, LHM, JC, and AD
 715 analyzed data; JC provided expertise for RNA-Seq; BF and SJB provided technical
 716 support and expertise for ChIP; RL, SJB, and AD wrote the manuscript; AD
 717 conceived and supervised the project.

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719

720 **DECLARATION OF INTEREST**

721 The authors declare no competing interests.

722

REFERENCES

- Akdemir F, Christich A, Sogame N, Chapo J, Abrams JM. 2007. p53 directs focused genomic responses in Drosophila. *Oncogene* **26**:5184–5193. doi:10.1038/sj.onc.1210328
- Anders S, Huber W. 2010. Differential expression analysis for sequence count data. *Genome Biol* **11**:R106. doi:10.1186/gb-2010-11-10-r106
- Atkins M, Potier D, Romanelli L, Jacobs J, Mach J, Hamaratoglu F, Aerts S, Halder G. 2016. An Ectopic Network of Transcription Factors Regulated by Hippo Signaling Drives Growth and Invasion of a Malignant Tumor Model. *Curr Biol* **26**:2101–2113. doi:10.1016/j.cub.2016.06.035
- Baillon L, Germani F, Rockel C, Hilchenbach J, Basler K. 2018. Xrp1 is a transcription factor required for cell competition-driven elimination of loser cells. *Sci Rep* **8**:17712. doi:10.1038/s41598-018-36277-4
- Banks L, Pim D, Thomas M. 2012. Human tumour viruses and the deregulation of cell polarity in cancer. *Nat Rev Cancer* **12**:877–886. doi:10.1038/nrc3400
- Bilder D, Schober M, Perrimon N. 2003. Integrated activity of PDZ protein complexes regulates epithelial polarity. *Nat Cell Biol* **5**:53–58. doi:10.1038/ncb897
- Blanco J, Cooper JC, Baker NE. 2020. Roles of C/EBP class bZip proteins in the growth and cell competition of Rp ('Minute') mutants in Drosophila. *Elife* **9**. doi:10.7554/eLife.50535
- Boulant L, Andersen D, Colombani J, Boone E, Léopold P. 2019. Inter-Organ Growth Coordination Is Mediated by the Xrp1-Dilp8 Axis in Drosophila. *Dev Cell* **49**:811–818.e4. doi:10.1016/j.devcel.2019.03.016
- Bray SJ. 2016. Notch signalling in context. *Nat Rev Mol Cell Biol* **17**:722–735. doi:10.1038/nrm.2016.94
- Brumby AM, Richardson HE. 2003. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. *EMBO J* **22**:5769–5779. doi:10.1093/emboj/cdg548
- Bunker BD, Nellimoottil TT, Boileau RM, Classen AK, Bilder D. 2015. The transcriptional response to tumorigenic polarity loss in Drosophila. *Elife* **4**. doi:10.7554/eLife.03189
- Coopman P, Djiane A. 2016. Adherens Junction and E-Cadherin complex regulation by epithelial polarity. *Cell Mol Life Sci* **73**:3535–3553. doi:10.1007/s00018-016-2260-8
- Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, Inui M, Montagner M, Parenti AR, Poletti A, Daidone MG, Dupont S, Basso G, Biciato S, Piccolo S. 2011. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* **147**:759–772. doi:10.1016/j.cell.2011.09.048
- Cordero JB, Macagno JP, Stefanatos RK, Strathdee KE, Cagan RL, Vidal M. 2010. Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. *Dev Cell* **18**:999–1011. doi:10.1016/j.devcel.2010.05.014
- Davie K, Jacobs J, Atkins M, Potier D, Christiaens V, Halder G, Aerts S. 2015. Discovery of transcription factors and regulatory regions driving in vivo tumor development by ATAC-seq and FAIRE-seq open chromatin profiling. *PLoS Genet* **11**:e1004994. doi:10.1371/journal.pgen.1004994
- Djiane A, Krejci A, Bernard F, Fexova S, Millen K, Bray SJ. 2013. Dissecting the mechanisms of Notch induced hyperplasia. *EMBO J* **32**:60–71. doi:10.1038/emboj.2012.326
- Doggett K, Turkel N, Willoughby LF, Ellul J, Murray MJ, Richardson HE, Brumby AM. 2015. BTB-Zinc Finger Oncogenes Are Required for Ras and Notch-Driven Tumorigenesis in Drosophila. *PLoS ONE* **10**:e0132987. doi:10.1371/journal.pone.0132987

Donohoe CD, Csordás G, Correia A, Jindra M, Klein C, Habermann B, Uhlirova M. 2018. Atf3 links loss of epithelial polarity to defects in cell differentiation and cytoarchitecture. *PLoS Genet* **14**:e1007241. doi:10.1371/journal.pgen.1007241

Francis MJ, Roche S, Cho MJ, Beall E, Min B, Panganiban RP, Rio DC. 2016. Drosophila IRBP bZIP heterodimer binds P-element DNA and affects hybrid dysgenesis. *Proc Natl Acad Sci USA* **113**:13003–13008. doi:10.1073/pnas.1613508113

Fre S, Huyghe M, Mourikis P, Robine S, Louvard D, Artavanis-Tsakonas S. 2005. Notch signals control the fate of immature progenitor cells in the intestine. *Nature* **435**:964–968. doi:10.1038/nature03589

Han W, Ming M, Zhao R, Pi J, Wu C, He Y-Y. 2012. Nrf1 CNC-bZIP protein promotes cell survival and nucleotide excision repair through maintaining glutathione homeostasis. *J Biol Chem* **287**:18788–18795. doi:10.1074/jbc.M112.363614

Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calton M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D. 2003. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* **11**:619–633. doi:10.1016/s1097-2765(03)00105-9

Herzig RP, Andersson U, Scarpulla RC. 2000. Dynein light chain interacts with NRF-1 and EWG, structurally and functionally related transcription factors from humans and drosophila. *J Cell Sci* **113 Pt 23**:4263–4273.

Ho DM, Pallavi SK, Artavanis-Tsakonas S. 2015. The Notch-mediated hyperplasia circuitry in Drosophila reveals a Src-JNK signaling axis. *Elife* **4**:e05996. doi:10.7554/eLife.05996

Huang L, Muthuswamy SK. 2010. Polarity protein alterations in carcinoma: a focus on emerging roles for polarity regulators. *Curr Opin Genet Dev* **20**:41–50. doi:10.1016/j.gde.2009.12.001

Huggins CJ, Mayekar MK, Martin N, Saylor KL, Gonit M, Jailwala P, Kasoji M, Haines DC, Quiñones OA, Johnson PF. 2015. C/EBPγ Is a Critical Regulator of Cellular Stress Response Networks through Heterodimerization with ATF4. *Mol Cell Biol* **36**:693–713. doi:10.1128/MCB.00911-15

Igaki T, Pagliarini RA, Xu T. 2006. Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila. *Curr Biol* **16**:1139–1146. doi:10.1016/j.cub.2006.04.042

Igaki T, Pastor-Pareja JC, Aonuma H, Miura M, Xu T. 2009. Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. *Dev Cell* **16**:458–465. doi:10.1016/j.devcel.2009.01.002

Ji Z, Kiparaki M, Folgado V, Kumar A, Blanco J, Rimesso G, Chuen J, Liu Y, Zheng D, Baker NE. 2019. Drosophila RpS12 controls translation, growth, and cell competition through Xrp1. *PLoS Genet* **15**:e1008513. doi:10.1371/journal.pgen.1008513

Katheder NS, Khezri R, O'Farrell F, Schultz SW, Jain A, Rahman MM, Schink KO, Theodossiou TA, Johansen T, Juhász G, Bilder D, Brech A, Stenmark H, Rusten TE. 2017. Microenvironmental autophagy promotes tumour growth. *Nature* **541**:417–420. doi:10.1038/nature20815

Külshammer E, Mundorf J, Kilinc M, Frommolt P, Wagle P, Uhlirova M. 2015. Interplay among Drosophila transcription factors Ets21c, Fos and Ftz-F1 drives JNK-mediated tumor malignancy. *Dis Model Mech* **8**:1279–1293. doi:10.1242/dmm.020719

Kusch T, Florens L, Macdonald WH, Swanson SK, Glaser RL, Yates JR, Abmayr SM, Washburn MP, Workman JL. 2004. Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. *Science* **306**:2084–2087. doi:10.1126/science.1103455

Lee C-H, Kiparaki M, Blanco J, Folgado V, Ji Z, Kumar A, Rimesso G, Baker NE. 2018. A Regulatory Response to Ribosomal Protein Mutations Controls Translation, Growth, and Cell Competition. *Dev Cell* **46**:456–469.e4. doi:10.1016/j.devcel.2018.07.003

Link N, Kurtz P, O'Neal M, Garcia-Hughes G, Abrams JM. 2013. A p53 enhancer region regulates target genes through chromatin conformations in cis and in trans. *Genes Dev* **27**:2433–2438. doi:10.1101/gad.225565.113

Loubiere V, Papadopoulos GL, Szabo Q, Martinez A-M, Cavalli G. 2020. Widespread activation of developmental gene expression characterized by PRC1-dependent chromatin looping. *Sci Adv* **6**:eaax4001. doi:10.1126/sciadv.aax4001

Maraver A, Fernández-Marcos PJ, Herranz D, Muñoz-Martin M, Gomez-Lopez G, Cañamero M, Mulero F, Megías D, Sanchez-Carbayo M, Shen J, Sanchez-Cespedes M, Palomero T, Ferrando A, Serrano M. 2012. Therapeutic effect of γ -secretase inhibition in KrasG12V-driven non-small cell lung carcinoma by derepression of DUSP1 and inhibition of ERK. *Cancer Cell* **22**:222–234. doi:10.1016/j.ccr.2012.06.014

McCaffrey LM, Montalbano J, Mihai C, Macara IG. 2012. Loss of the Par3 polarity protein promotes breast tumorigenesis and metastasis. *Cancer Cell* **22**:601–614. doi:10.1016/j.ccr.2012.10.003

Muzzopappa M, Murcia L, Milán M. 2017. Feedback amplification loop drives malignant growth in epithelial tissues. *Proc Natl Acad Sci USA* **114**:E7291–E7300. doi:10.1073/pnas.1701791114

Ntziachristos P, Lim JS, Sage J, Aifantis I. 2014. From fly wings to targeted cancer therapies: a centennial for notch signaling. *Cancer Cell* **25**:318–334. doi:10.1016/j.ccr.2014.02.018

Ohsawa S, Sugimura K, Takino K, Xu T, Miyawaki A, Igaki T. 2011. Elimination of oncogenic neighbors by JNK-mediated engulfment in *Drosophila*. *Dev Cell* **20**:315–328. doi:10.1016/j.devcel.2011.02.007

Pagliarini RA, Xu T. 2003. A genetic screen in *Drosophila* for metastatic behavior. *Science* **302**:1227–1231. doi:10.1126/science.1088474

Pascual J, Jacobs J, Sansores-Garcia L, Natarajan M, Zeitlinger J, Aerts S, Halder G, Hamaratoglu F. 2017. Hippo Reprograms the Transcriptional Response to Ras Signaling. *Dev Cell* **42**:667–680.e4. doi:10.1016/j.devcel.2017.08.013

Pinal N, Martín M, Medina I, Morata G. 2018. Short-term activation of the Jun N-terminal kinase pathway in apoptosis-deficient cells of *Drosophila* induces tumorigenesis. *Nat Commun* **9**:1541. doi:10.1038/s41467-018-04000-6

Ranganathan P, Weaver KL, Capobianco AJ. 2011. Notch signalling in solid tumours: a little bit of everything but not all the time. *Nat Rev Cancer* **11**:338–351. doi:10.1038/nrc3035

Reddy KL, Rovani MK, Wohlwill A, Katzen A, Storti RV. 2006. The *Drosophila* Par domain protein I gene, Pdp1, is a regulator of larval growth, mitosis and endoreplication. *Dev Biol* **289**:100–114. doi:10.1016/j.ydbio.2005.10.042

Reinke AW, Baek J, Ashenberg O, Keating AE. 2013. Networks of bZIP protein-protein interactions diversified over a billion years of evolution. *Science* **340**:730–734. doi:10.1126/science.1233465

St Johnston D, Ahringer J. 2010. Cell polarity in eggs and epithelia: parallels and diversity. *Cell* **141**:757–774. doi:10.1016/j.cell.2010.05.011

Sykietis GP, Bohmann D. 2008. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in *Drosophila*. *Dev Cell* **14**:76–85. doi:10.1016/j.devcel.2007.12.002

Thurmond J, Goodman JL, Strelets VB, Attrill H, Gramates LS, Marygold SJ, Matthews BB, Millburn G, Antonazzo G, Trovisco V, Kaufman TC, Calvi BR, FlyBase Consortium. 2019. FlyBase 2.0: the next generation. *Nucleic Acids Res* **47**:D759–D765. doi:10.1093/nar/gky1003

Toedling J, Skylar O, Sklyar O, Krueger T, Fischer JJ, Sperling S, Huber W. 2007. Ringo--an R/Bioconductor package for analyzing ChIP-chip readouts. *BMC Bioinformatics* **8**:221. doi:10.1186/1471-2105-8-221

882 Toggweiler J, Willecke M, Basler K. 2016. The transcription factor Ets21C drives tumor
883 growth by cooperating with AP-1. *Sci Rep* **6**:34725. doi:10.1038/srep34725
884 Turkel N, Sahota VK, Bolden JE, Goulding KR, Doggett K, Willoughby LF, Blanco E,
885 Martin-Blanco E, Corominas M, Ellul J, Aigaki T, Richardson HE, Brumby AM.
886 2013. The BTB-zinc finger transcription factor abrupt acts as an epithelial
887 oncogene in *Drosophila melanogaster* through maintaining a progenitor-like cell
888 state. *PLoS Genet* **9**:e1003627. doi:10.1371/journal.pgen.1003627
889 Uhlirova M, Bohmann D. 2006. JNK- and Fos-regulated Mmp1 expression cooperates
890 with Ras to induce invasive tumors in *Drosophila*. *EMBO J* **25**:5294–5304.
891 doi:10.1038/sj.emboj.7601401
892 Uhlirova M, Jasper H, Bohmann D. 2005. Non-cell-autonomous induction of tissue
893 overgrowth by JNK/Ras cooperation in a *Drosophila* tumor model. *Proc Natl*
894 *Acad Sci USA* **102**:13123–13128. doi:10.1073/pnas.0504170102
895 Wu M, Pastor-Pareja JC, Xu T. 2010. Interaction between Ras(V12) and scribbled clones
896 induces tumour growth and invasion. *Nature* **463**:545–548.
897 doi:10.1038/nature08702
898 Xue B, Krishnamurthy K, Allred DC, Muthuswamy SK. 2013. Loss of Par3 promotes
899 breast cancer metastasis by compromising cell-cell cohesion. *Nat Cell Biol*
900 **15**:189–200. doi:10.1038/ncb2663
901 Ye J, Rawson RB, Komuro R, Chen X, Davé UP, Prywes R, Brown MS, Goldstein JL. 2000.
902 ER stress induces cleavage of membrane-bound ATF6 by the same proteases
903 that process SREBPs. *Mol Cell* **6**:1355–1364. doi:10.1016/s1097-
904 2765(00)00133-7
905
906

FIGURE LEGENDS

Figure 1. Notch-based neoplastic growth paradigms in *Drosophila* wing discs

A-D. 3rd instar wing imaginal discs at precisely 5 days after egg-laying either wild-type (WT; A), overexpressing activated Notch (N; B), mutant for *scrib* (S; C), or combining overexpressed Notch and *scrib* mutation (NS; D) and marked for E-Cad (blue) and Mmp1 (red). **A,B&D.** MARCM clones (positively marked by GFP; green) of the indicated genotypes: expressing only GFP (A; WT), expressing Nicd & GFP (B; N), and expressing Nicd & GFP and mutant for *scrib* (C; NS). **C.** Discs fully mutant for *scrib*.

E-F. Differentially expressed genes as compared to WT in the different growth paradigms N (green), S (red), and NS (blue) identified by RNA-Seq. This color code, green for N, red for S, and blue for NS is used in all figures. **E.** Heatmap of gene expressions after unsupervised clustering. **F.** Venn diagram of up-regulated and down-regulated genes in N, S, and NS.

G. 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.

Figure 2. GO term analysis of the N, S, and NS transcriptomes

A. Enrichment diagram as measured by adjusted p-value for selected GO terms (full list as supplemental material) and represented as bars for N (green), S (red), and NS (blue). GO terms color reflect whether they are shared or specific: shared by all (black), common N&S (orange: mix of green and red), common N&NS (dark green: mix of green and blue), common S&NS (purple: mix of red and blue).

B. Venn diagram showing the domains of overlap of GO terms identified (significantly enriched) in N, S, and NS.

Figure 3. Polarity loss redirects the transcriptional output of Notch during neoplastic growth

A. Experimental set-up to identify the Notch Direct Targets genes (NDTs): Genes up-regulated in N, S, or NS (Transcriptomic), and located within 20kb of a Su(H) binding site (ChIP).

940 **B.** Venn diagram showing the NDTs overlap in N, S, and NS, showing core Notch
 941 responses, but also significant condition specific NDTs.

942 **C.** Overlap of the Su(H) binding sites identified by ChIP in N, S, and NS, showing
 943 that almost all S and NS Su(H) peaks are also found in N. The overlap is shown in
 944 white. Numbers are slightly different because in the Su(H) peaks calling protocol, in
 945 some rare cases, some peaks can be split between conditions where one peak in one
 946 condition would overlap with two peaks in the other.

947 **D.** Genome Viewer snapshots of several NDTs (shown in purple) such as the NS
 948 specific *Act87E* and *Wnt10* (but also *yellow-e* and *Ir87a*), the common *Ets21C*, and
 949 the N/NS NDT *upd3*. For each condition, the Su(H) ChIP enrichment is shown in the
 950 upper lane, and the Su(H) peaks identified are represented by the blocks underneath.

951

952 **Figure 4. Identification of potential transcriptional modules mediating N, S, and**
 953 **NS growth**

954 **A.** Venn diagram of significant transcription factors (TFs) identified by iRegulon as
 955 potential key mediators for the expression of the N (in the green circle), S (red circle),
 956 and NS (blue circle) up-regulated genes. Fed with lists of co-regulated genes, and
 957 analyzing the genomic features in the vicinity of the transcription start sites of these
 958 genes, iRegulon identifies potential groups of TFs and DNA-binding factors, that are
 959 enriched in the dataset of regulatory sequences, and could thus represent potential
 960 mediators of the N, S, and NS transcriptomes. TFs were color-coded according to
 961 their molecular class and/or belonging to the similar regulon (see supplemental tables
 962 S8-15). Numbers represent the number of TFs identified. See also Figure S2 for the
 963 iRegulon analyses of the NDTs, and the detailed lists of both iRegulon analyses in
 964 Supplemental tables S8 to S15.

965 **B.** Heat map for the expression of some of the transcription factors identified by
 966 iRegulon and grouped as “oncogenic module”, “basic Leucine Zippers”, “DNA
 967 damage and genome integrity”.

968

969 **Figure 5. Neoplastic growth is mediated by the “oncogenic module” and by a**
 970 **diverse network of bZIP transcription factors including Xrp1**

971 **A-H.** 3rd instar wing imaginal discs expressing *GFP*, an activated form of Notch
 972 (*Nicd*) and an RNAi for *scrib* under the control of the *Bx-Gal4* driver (dorsal wing
 973 pouch) and stained for GFP (green, white A'-H') to assess tissue overgrowth and for

974 Mmp1 (red, white A''-H'') to assess tissue invasiveness. Discs also expressed under
 975 *Bx-Gal4* control the either *UAS Bsk DN* (B) or the indicated RNAi constructs:
 976 *w[HMS00045]* (A; Ctrl), *yki[KK109756]* (C), *crc[JF02007]* (D),
 977 *CG6272[HMS00057]* (E), *Xrp1[HMJ21189]* (F), *maf-S[HMS02020]* (G),
 978 *pdp1[HMS02030]* (H). Representative discs are shown.
 979 **I-K**. Quantification of the overgrowth of the GFP territory in the indicated genotypes.
 980 Results are shown as percentage of discs. In blue are shown the discs smaller than the
 981 average of controls (size < Average size of controls – 1.5x StDev of controls). In
 982 green are shown discs that are the same size as the controls average or bigger. Results
 983 for the “DNA damage module” in **I**, for the “oncogenic module” in **J**, and for the
 984 “AP-1 and bZIP module” in **K**. Green arrowheads indicate modification. Star indicate
 985 a genotype with misshapen and “sick” discs. Sample size 13 – 17 discs.
 986 For more details of RNAi lines either from the TRiP collection (labelled with trip
 987 superscript) or from the Vienna collection (labelled with KK superscript) are provided
 988 in supplemental information.
 989 **I'-K'**. Quantification of the Mmp1 intensity in the indicated genotypes. Results are
 990 shown as percentage where discs were classified to fall in three categories: High
 991 Mmp1 staining intensity (red, similar to that shown in A''), Low Mmp1 staining
 992 intensity (blue, similar to that shown in C or H), No Mmp1 staining (light blue,
 993 similar to that shown in B''). Results for the “DNA damage module” in **I'**, for the
 994 “oncogenic module” in **J'**, and for the “AP-1 and bZIP module” in **K'**. Red
 995 arrowheads indicate modification. Star indicate a genotype with misshapen and “sick”
 996 discs. Sample size 13 – 17 discs.
 997 For more details of RNAi lines either from the TRiP collection (labelled with trip
 998 superscript) or from the Vienna collection (labelled with KK superscript) are provided
 999 in supplemental information.

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL MATERIAL AND METHODS

Drosophila genetics

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. 5I-K&I'-K' and whether pictures are shown in Fig. 5A-H. TRiP collection lines have a stock BL#, and Vienna collection lines have a stock v#.

Gene	RNAi ID	Stock #	Label in Fig. 5 I-K and I'-K'	Discs shown in Fig. 5 A-H
Atf-2	HMC05118	BL#60124	Atf2 trip	
Atf3	JF02303	BL#26741	Atf3 trip	
Atf6	JF02109	BL#26211	Atf6 trip	
ATM/tefu	KK100008	v#108074	ATM KK	
ATR/mei-P26	HMC04662	BL#57268	ATR trip	
CG6272	KK110056	v#101871	CG6272 KK	
CG6272	HMS00057	BL#33652	CG6272 trip	Fig. 5E
CG7786	HMC05169	BL#62162	CG7786 trip	
cnc	HMS00650	BL#32863	cnc trip	
crc	JF02007	BL#25985	crc trip	Fig. 5D
CrebB-17A	HMJ30249	BL#63681	CrebB-17A trip	
Ets21C	HMS01989	BL#39069	Ets21C trip	
ftz-f1	KK108995	v#104463	ftz-f1 KK	
gt	HMS01105	BL#34631	gt trip	
Jra	JF01184	BL#31595	Jra trip	
kay	HMS00254	BL#33379	kay trip	
maf-S	HMS02020	BL#40853	maf-S trip	Fig. 5G
p53	HMS02286	BL#41720	p53 trip	
pdp1	HMS02030	BL#40863	pdp1 trip	Fig. 5H

Stat92E	HMS00035	BL#33637	Stat92E trip	
vri	HMS02029	BL#40862	vri trip	
w	HMS00045	BL#33644	Ctrl	Fig. 5A
Xrp1	HMS00053	BL#34521	Xrp1 trip#1	
Xrp1	HMJ21189	BL#51054	Xrp1 trip#2	Fig. 5F
Xrp1	HMJ22533	BL#60356	Xrp1 trip#3	
yki	KK109756	v#111001	yki KK	Fig. 5C

1011

1012

1013 **Primers**

1014 qPCR primers:

1015 Act5C_F: GAGCGCGGTTACTCTTTCAC
1016 Act5C_R: ACTTCTCCAACGAGGAGCTG
1017 Act87E_F: GTCCACCGCAAGTGCTTCTA
1018 Act87E_R: TTTCTTTGGATGGCAGGGCA
1019 Atf3_F: CAGCATGGCAACATTGGGAC
1020 Atf3_R: ATGAAGGCAGTGGCTGAGTC
1021 Diap1_F: CAGCCACACGCATCTTCAAC
1022 Diap1_R: ACTTTGTCACAGAGGAGGCG
1023 E2f1_F: ACAGAATCCTCGCCTCCAAC
1024 E2f1_R: GACTGCTGCCGTAGCCTATT
1025 Ets21C_F: CTGCTCGCTGATTCGTCCAA
1026 Ets21C_R: TAGGCATACCGCTTTCCGTG
1027 ftz-f1_F: ATTCCTGGTTCGGACATGCTT
1028 ftz-f1_R: TTCATGCAGACATAGTCGCCC
1029 mxc_F: ACTAGAGGAGGAGCAGCGAA
1030 mxc_R: CTAGTGGACAGCGGCGTATT
1031 p38a_F: TACGGACAGGTGTCAAAGGC
1032 p38a_R: CAGCGATCCATTAGCGGGAT
1033 p53_F: TGCGTGTGTTCTTTGCTTC
1034 p53_R: GTTCAGGGGGACTACAACGG
1035 puc_F: ATTGACCTCGCCGCCAATTA
1036 puc_R: ATTCCGCTTGAACAGAGCCA

1037 sd_F: AGGGTCCACAGAATGCGTTT
 1038 sd_R: TCGCTTTCCACCTTCTCCAC
 1039 Sdr_F: CGCTCCCTCAATCCCAAAGT
 1040 Sdr_R: ACAACGTCCATCAGCCAGTT
 1041 Ser_F: GCACGAATCTCTGGTGTGGA
 1042 Ser_R: TAGATTTGGCTGGCAGTCGG
 1043 wg_F: GCAGTCTGGTCTGGTCTACG
 1044 wg_R: ATTGTCGGGTTTCAGTTGGA
 1045 Wnt10_F: AATGGCATCGGTGGAAGTGT
 1046 Wnt10_R: CAGCGTCTTGCGATTGATGG
 1047
 1048 qChIP primers:
 1049 E(spl)mβ_F: AAGTCGGAGCTTTGAATGAG
 1050 E(spl)mβ_R: CAAGTCATTTTATTGCCCTCAC
 1051 E(spl)m5_F: GTTCCGCAGGTCCAGTTAC
 1052 E(spl)m5_R: GTTTGATGTTACGCTGCTG
 1053 white_F: CGAAGGACGTTGACACATTG
 1054 white_R: GAATTGCCGCTTTTCTCAC
 1055 DDC_F: AAGTGGGATTTGCCAGTGAC
 1056 DDC_R: TGCTGGTGAACCTTTGACTGC
 1057 CG42808_F: CTCGTTAAGAGCAACTGCGA
 1058 CG42808_R: GTGAGAACTCCGAATCGAGG
 1059 CG6191_F: CGAAAAATGCGGACGATTCC
 1060 CG6191_R: CCCACCAATCTAGGGTTTCA
 1061 Ilp8_F: TCATCTCCGGTGTCTGACTT
 1062 Ilp8_R: AAAGAATTGGCTGCGGAAGA

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1065 **SUPPLEMENTAL FIGURE LEGENDS**

1066 **Figure S1. Features of the Notch Direct Targets (NDTs) in N, S, and NS (relates**
 1067 **to Fig. 3)**

1068 **A.** Genome-wide localization of the Su(H) ChIP enrichment peaks with respect to
 1069 genome features, showing that the major changes were found in NS where Su(H)

1070 “peaks” were more prevalent in introns but less prevalent at promoter regions than in
1071 N and S.

1072 **B.** Heatmaps for the expression of the different NDTs in WT, N, S, and NS. From left
1073 to right are presented the N, S, NS, and finally All NDTs, highlighting that NDTs
1074 could be transcriptionally up-regulated in more than in one condition.

1075 **C.** Genome browser view of the whole left arm of the 2nd chromosome, and showing
1076 the Su(H) ChIP enrichment (upper rows)) and the intervals called as Su(H) peaks
1077 (lower rows) in N (green), NS (blue), and S (red). Note the higher number of peaks in
1078 N, and the rarity of NS, or S peaks not found in N.

1079

1080 **Figure S2. Identification of potential transcriptional modules mediating N, S,
1081 and NS growth (relates to Fig. 4)**

1082 **A.** Venn diagram of significant transcription factors identified by iRegulon in N, S,
1083 and NS Notch Direct Targets, and color-coded according to their molecular class.
1084 Numbers represent the number of transcription factors identified in each group. See
1085 also the detailed lists of all iRegulon analyses in Supplemental tables S8 to S15.

1086

1087

1088 **SUPPLEMENTAL TABLES**

1089 **Table S1-3.** Differentially expressed genes in N, S, and NS identified by DESeq
1090 (related to Fig. 1). Columns are:

1091 FBgn_ID: Unique FlyBase gene ID

1092 Symbol: Current FlyBase gene symbol

1093 qval: adjusted p-value for multiple testing

1094 logFC: log₂ of the Fold Change “Condition N, S, or NS” / “Control WT”

1095

1096 **Table S4.** Gene Ontology (GO) enrichment in N, S, and NS (related to Fig. 2).

1097 Columns are:

1098 experiment: type of comparison

1099 GO_term: GO term number

1100 description: GO term description

1101 intersect.size: number of genes in query within the GO term

1102 n.pw: total number of genes within the given GO term

1103 pval: p value after hypergeometric test

1104 qval: adjusted pvalue for multiple testing
 1105 genes: genes in query falling in the specific GO term
 1106
 1107 **Table S5.** Curated Gene Ontology (GO) enrichment in N, S, and NS (related to Fig.
 1108 2). Columns are:
 1109 experiment: type of comparison
 1110 GO_term: GO term number
 1111 description: GO term description
 1112 intersect.size: number of genes in query within the GO term
 1113 n.pw: total number of genes within the given GO term
 1114 pval: p value after hypergeometric test
 1115 qval: adjusted pvalue for multiple testing
 1116 genes: genes in query falling in the specific GO term
 1117
 1118 **Table S6.** Su(H) ChIP enrichment peaks coordinates in N, S, and NS (related to Fig.
 1119 3). Columns are:
 1120 Exp: N, S, or NS
 1121 Chr: Chromosome arm
 1122 MIN: smallest peak coordinate
 1123 MAX: biggest peak coordinate
 1124
 1125
 1126 **Table S7.** All Notch Direct Targets (NDTs) ordered by genomic position. This table
 1127 includes an indication whether the genes are transcriptionally upregulated or have a
 1128 Su(H) peak in the vicinity in each N, S, and NS condition. Columns are:
 1129 N/NS/S: NDT in the corresponding condition
 1130 Type: NDT in different conditions.
 1131 FBgn_ID: Unique FlyBase gene ID
 1132 SYMBOL: Current FlyBase gene symbol
 1133 K_ARM: Chromosome arm location of the gene
 1134 MIN (gene pos): smallest gene coordinate
 1135 MAX (gene pos): biggest gene coordinate
 1136 STRAND: +1 or -1
 1137 N Fold: Log2 Fold Change in gene expression N/WT (n.s. not significant)

1138 N ChIP: Su(H) ChIP enrichment peak within 20kb in N (green yes, red no)
 1139 NS Fold: Log2 Fold Change in gene expression NS/WT (n.s. not significant)
 1140 NS ChIP: Su(H) ChIP enrichment peak within 20kb in NS (green yes, red no)
 1141 S Fold: Log2 Fold Change in gene expression S/WT (n.s. not significant)
 1142 S ChIP: Su(H) ChIP enrichment peak within 20kb in S (green yes, red no)
 1143
 1144 **Table S8-10.** iRegulon analyses of the significantly upregulated genes in N, S, and
 1145 NS (related to Fig. 4). Analyses were performed using the 6K-PWM and 10kb
 1146 upstream and downstream set-ups.
 1147
 1148 **Table S11.** Curated iRegulon analyses corresponding to Tables S8-10 (related to Fig.
 1149 4)
 1150
 1151 **Table S12-14.** iRegulon analyses of the Notch Direct Targets in N, S, and NS (related
 1152 to Fig. S2). Analyses were performed using the 6K-PWM and 10kb upstream and
 1153 downstream set-ups.
 1154
 1155 **Table S15.** Curated iRegulon analyses corresponding to Tables S12-14 (related to
 1156 Fig. S2)
 1157
 1158 **Table S16.** Xrp1 target genes. Xrp1 regulated genes identified in Lee et al. (2018)
 1159 (positively and negatively) and in Baillon et al. (2018). In bold are highlighted genes
 1160 found in both studies. Columns are:
 1161 FBgn_ID: Unique FlyBase gene ID
 1162 Symbol: Current FlyBase gene symbol

A

B

A

B

C

D

A

B

A Localisation of the Su(H) binding on the genome in each condition (in %)

B Heat-maps of the expression of N, S, or NS NDTs (left to right) in each condition

C Genome browser view of chromosome 2L with Su(H) peak calling in N, S, and NS

A

N

N / NS

NS

N / S / NS

N / S

S/NS

S

fd102C	maf-S
CG8079	CrebB-17A

retn lola