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**The *Drosophila* SWI/SNF chromatin-remodeling complexes BAP and PBAP play separate roles in regulating growth and cell fate during regeneration**

Running Title: SWI/SNF regulates regeneration

Yuan Tian<sup>1</sup> and Rachel K. Smith-Bolton<sup>1,2\*</sup>

<sup>1</sup>Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

<sup>2</sup>Carl R. Woese Institute for Genomic Biology

\*Author for correspondence: [rsbolton@illinois.edu](mailto:rsbolton@illinois.edu)

**Key words**

regeneration, chromatin, SWI/SNF complexes, cell fate, *Drosophila*, wing imaginal disc

27 **Summary statement**

28 During regeneration of the *Drosophila* wing disc, the SWI/SNF PBAP complex is  
29 required for regenerative growth and expression of JNK signaling targets, while  
30 the BAP complex maintains posterior cell fate.

31

32 **Abstract**

33 To regenerate, damaged tissue must heal the wound, regrow to the proper size,  
34 replace the correct cell types, and return to the normal gene-expression program.  
35 However, the mechanisms that temporally and spatially control the activation or  
36 repression of important genes during regeneration are not fully understood. To  
37 determine the role that chromatin modifiers play in regulating gene expression af-  
38 ter tissue damage, we induced ablation in *Drosophila* imaginal wing discs, and  
39 screened for chromatin regulators that are required for epithelial tissue regenera-  
40 tion. Here we show that many of these genes are indeed important for promoting  
41 or constraining regeneration. Specifically, the two SWI/SNF chromatin-remodel-  
42 ing complexes play distinct roles in regulating different aspects of regeneration.  
43 The PBAP complex regulates regenerative growth and developmental timing,  
44 and is required for the expression of JNK signaling targets and the growth pro-  
45 moter *Myc*. By contrast, the BAP complex ensures correct patterning and cell  
46 fate by stabilizing expression of the posterior gene *engrailed*. Thus, both  
47 SWI/SNF complexes are essential for proper gene expression during tissue re-  
48 generation, but they play distinct roles in regulating growth and cell fate.

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## 53 **Introduction**

54 Regeneration is a complex yet highly elegant process that some organisms can  
55 use to recognize, repair and replace missing or damaged tissue. Imaginal disc  
56 repair in *Drosophila* is a good model system for understanding regeneration due  
57 to the high capacity of these tissues to regrow and restore complex patterning, as  
58 well as the genetic tools available in this model organism (Hariharan and Serras,  
59 2017). Regeneration requires the coordinated expression of genes that regulate  
60 the sensing of tissue damage, induction of regenerative growth, repatterning of  
61 the tissue, and coordination of regeneration with developmental timing. Initiation  
62 of regeneration in imaginal discs requires known signaling pathways such as the  
63 ROS, JNK, Wg, p38, Jak/STAT, and Hippo pathways (Bergantinos et al., 2010;  
64 Bosch et al., 2008; Grusche et al., 2011; Katsuyama et al., 2015; Santabárbara-  
65 Ruiz et al., 2015; Schubiger et al., 2010; Smith-Bolton et al., 2009; Sun and Ir-  
66 vine, 2011). These pathways activate many regeneration genes, such as the  
67 growth promoter Myc (Smith-Bolton et al., 2009) and the hormone-like peptide  
68 *ilp8*, which delays pupariation after imaginal disc damage (Colombani et al.,  
69 2012; Garelli et al., 2012). However, misregulation of these signals can impair re-  
70 generation. For example, elevated levels of JNK signaling can induce patterning  
71 defects in the posterior of the wing (Schuster and Smith-Bolton, 2015), and ele-  
72 vated ROS levels can suppress JNK activity and regenerative growth (Brock et  
73 al., 2017). While the signals that initiate regeneration have been extensively stud-  
74 ied, regulation of regeneration gene expression in response to tissue damage is  
75 not fully understood.

76

77 Such regulation could occur through chromatin modification. In *Drosophila*, chro-  
78 matin modifiers include the repressive complexes PRC1 and PRC2, the

79 activating complexes TAC1, COMPASS and COMPASS-like, and the SWI/SNF  
80 chromatin remodelers BAP and PBAP (Kassis et al., 2017). PRC2 carries out tri-  
81 methylation of histone H3 at lysine 27, recruiting PRC1 to repress transcription of  
82 nearby genes. COMPASS-like and COMPASS carry out histone H3 lysine 4  
83 monomethylation and di- and trimethylation, respectively, thereby activating ex-  
84 pression of nearby genes. TAC1 acetylates histone H3 lysine 27, also supporting  
85 activation of gene transcription. BAP and PBAP alter or move nucleosomes to fa-  
86 cilitate binding of transcription factors and chromatin modifiers. Rapid changes in  
87 gene expression induced by these complexes may help facilitate a damaged tis-  
88 sue's regenerative response.

89

90 A few chromatin modifiers and histone modifications have been reported to be  
91 important for regulating regeneration of *Xenopus* tadpole tails, mouse pancreas  
92 and liver, zebrafish fins, and *Drosophila* imaginal discs (Blanco et al., 2010; Fu-  
93 kuda et al., 2012; Jin et al., 2015; Pfefferli et al., 2014; Scimone et al., 2010;  
94 Skinner et al., 2015; Stewart et al., 2009; Tseng et al., 2011; Wang et al., 2008).  
95 Furthermore, components of *Drosophila* and mouse SWI/SNF complexes regu-  
96 late regeneration in the *Drosophila* midgut and mouse skin, liver, and ear (Jin et  
97 al., 2013; Sun et al., 2016; Xiong et al., 2013). However, little is known about how  
98 these complexes alter gene expression, signaling, and cellular behavior to regu-  
99 late regeneration. Importantly, genome-wide analysis of chromatin state after  
100 *Drosophila* imaginal disc damage revealed changes in chromatin around a large  
101 set of genes, including known regeneration genes (Vizcaya-Molina et al., 2018).  
102 Thus, chromatin modifiers likely play a key role in regulating activation of the re-  
103 generation program. However, it is unclear whether all regeneration genes are  
104 coordinately regulated in the same manner, or whether specific chromatin

105 modification complexes target different subsets of genes that respond to tissue  
106 damage.

107

108 To probe the role of chromatin modifiers in tissue regeneration systematically, we  
109 assembled a collection of pre-existing *Drosophila* mutants and RNAi lines target-  
110 ing components of these complexes as well as other genes that regulate chroma-  
111 tin, and screened these lines for regeneration defects using the *Drosophila* wing  
112 imaginal disc. We used a spatially and temporally controllable tissue-ablation  
113 method that uses transgenic tools to induce tissue damage only in the wing pri-  
114 mordium (Smith-Bolton et al., 2009). This method ablates 94% of the wing pri-  
115 mordium on average at the early third instar and allows the damaged wing discs  
116 to regenerate *in situ*. Previous genetic screens using this tissue ablation method  
117 have identified genes critical for regulating different aspects of regeneration, such  
118 as *taranis*, *trithorax*, and *cap-n-collar*, demonstrating its efficacy in finding regen-  
119 eration genes (Brock et al., 2017; Schuster and Smith-Bolton, 2015; Skinner et  
120 al., 2015).

121

122 Through this targeted genetic screen of chromatin regulators we found that muta-  
123 tions in *Drosophila* SWI/SNF components caused striking regeneration defects.  
124 The SWI/SNF complexes are conserved multi-subunit protein complexes that ac-  
125 tivate or repress gene expression (Wilson and Roberts, 2011) by using the en-  
126 ergy from ATP hydrolysis to disrupt histone-DNA contacts and remodel nucleo-  
127 some structure and position (Côté et al., 1994; Kwon et al., 1994). Brm is the  
128 only ATPase of the SWI/SNF complexes in *Drosophila* (Kassis et al., 2017;  
129 Tamkun et al., 1992). Moira (Mor) serves as the core scaffold of the complexes  
130 (Mashtalir et al., 2018). Other components contain domains involved in protein-

131 protein interactions, protein-DNA interactions, or interactions with modified his-  
132 tones (Hargreaves and Crabtree, 2011). There are two subtypes of SWI/SNF in  
133 *Drosophila*: the Brahma-associated proteins (BAP) and the Polybromo-associ-  
134 ated BAP (PBAP) remodeling complexes (Collins and Treisman, 2000;  
135 Mohrmann et al., 2004). They share common core components, including Brm,  
136 Snr1, Mor, Bap55, Bap60, Bap111 and Actin (Mohrmann et al., 2004), but con-  
137 tain different signature proteins. The PBAP complex is defined by the compo-  
138 nents Bap170, Polybromo and Sayp (Mohrmann et al., 2004; Chalkley et al.,  
139 2008). Osa defines the BAP complex (Collins et al., 1999; Vázquez et al., 1999).

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141 Here we show that the SWI/SNF complexes BAP and PBAP are required for re-  
142 generation, and that the two complexes play distinct roles. The PBAP complex is  
143 important for activation of JNK signaling targets such as *ilp8* to delay metamor-  
144 phosis and allow enough time for the damaged tissue to regrow, and for expres-  
145 sion of *myc* to drive regenerative growth. By contrast, the BAP complex is not re-  
146 quired for regenerative growth, but instead functions to prevent changes in cell  
147 fate induced by tissue damage through stabilizing expression of the posterior  
148 identity gene *engrailed*. Thus, different aspects of the regeneration program are  
149 regulated independently by distinct chromatin regulators.

150

## 151 **Materials and Methods**

### 152 **Fly stocks**

153 The following fly stocks were obtained for this study. In some cases they were re-  
154 balanced before performing experiments: *w<sup>1118</sup>*;, *rnGAL4*, *UAS-rpr*, *tub-*  
155 *GAL80<sup>ts</sup>/TM6B*, *tubGAL80* (Smith-Bolton et al., 2009), *w<sup>1118</sup>* (Wild type), *w<sup>\*</sup>*;  
156 *P{neoFRT}82B osa<sup>308</sup>/TM6B*, *Tb<sup>1</sup>* (Bloomington *Drosophila* stock center,

157 BL#5949) (Treisman et al., 1997),  $w^*$ ; *Bap170*<sup>Δ135/T(2;3)</sup>*SM6a-TM6B*, *Tb*<sup>1</sup> was a  
158 gift from Jessica E. Treisman (Carrera et al., 2008), *brm*<sup>2</sup> *e*<sup>s</sup> *ca*<sup>1/TM6B</sup>, *Sb*<sup>1</sup> *Tb*<sup>1</sup>  
159 *ca*<sup>1</sup> (BL#3619) (Kennison and Tamkun, 1988), *mor*<sup>1/TM6B</sup>, *Tb*<sup>1</sup> (BL#3615) (Ken-  
160 nison and Tamkun, 1988), *y*<sup>1</sup> *w*<sup>1</sup>; *P{neoFRT}40A P{FRT(*w*<sup>hs</sup>)}G13 *cn*<sup>1</sup> *PBac{SAs-*  
161 *topDsRed}Bap55*<sup>LL05955</sup> *bw*<sup>1/CyO</sup>, *bw*<sup>1</sup> (BL#34495) (Schuldiner et al., 2008),  
162 *bap111* RNAi (Vienna *Drosophila* Resource Center, VDRC#104361), control  
163 RNAi background (VDRC#15293) *bap60* RNAi (VDRC#12673), *brm* RNAi  
164 (VDRC#37721), *P{PZ}tara*<sup>03881</sup> *ry*<sup>506/TM3</sup>, *ry*<sup>RK</sup> *Sb*<sup>1</sup> *Ser*<sup>1</sup> (BL#11613) (Gutierrez,  
165 2003), *UAS-tara* was a gift from Michael Cleary (Manansala et al., 2013), *TRE-*  
166 *Red* was a gift from Dirk Bohmann (Chatterjee and Bohmann, 2012). *mor*<sup>2</sup>, *mor*<sup>11</sup>  
167 and *mor*<sup>12</sup> alleles were gifts from James Kennison (Kennison and Tamkun,  
168 1988), *snr*<sup>1E2</sup> and *snr*<sup>1SR21</sup> alleles were gifts from Andrew Dingwall (Zrally et al.,  
169 2003).*

170 The mutants and RNA interference lines in Table S1 used for the chromatin regu-  
171 lator screen were:

172 *st*<sup>1</sup> *in*<sup>1</sup> *knj*<sup>iri-1</sup> *Scr*<sup>W</sup> *Pc*<sup>3/TM3</sup>, *Sb*<sup>1</sup> *Ser*<sup>1</sup> (BL#3399),  
173 *cn*<sup>1</sup> *Psc*<sup>1</sup> *bw*<sup>1</sup> *sp*<sup>1/CyO</sup> (BL#4200),  
174 *y*<sup>1</sup>  $w^*$ ; *P{neoFRT}42D Psc*<sup>e24/SM6b</sup>, *P{eve-lacZ8.0}SB1* (BL#24155),  
175  $w^*$ ; *P{neoFRT}82B Abd-B*<sup>Mcp-1</sup> *Sce*<sup>1/TM6C</sup>, *Sb*<sup>1</sup> *Tb*<sup>1</sup> (BL#24618),  
176  $w^*$ ; *P{neoFRT}82B Scm*<sup>D1/TM6C</sup>, *Sb*<sup>1</sup> *Tb*<sup>1</sup> (BL#24158),  
177  $w^*$ ; *E(z)*<sup>731</sup> *P{1xFRT.G}2A/TM6C*, *Sb*<sup>1</sup> *Tb*<sup>1</sup> (BL#24470),  
178  $w^*$ ; *Su(z)*<sup>122</sup> *P{FRT(*w*<sup>hs</sup>)}2A/TM6C*, *Sb*<sup>1</sup> *Tb*<sup>1</sup> (BL#24159),  
179 *esc*<sup>21</sup> *b*<sup>1</sup> *cn*<sup>1/ln(2LR)Gla</sup>, *wg*<sup>Gla-1</sup>; *ca*<sup>1</sup> *awd*<sup>K</sup> (BL#3623),  
180 *y*<sup>1</sup> *w*<sup>67c23</sup>; *P{wHy}Caf1-55*<sup>DG25308</sup> (BL#21275),  
181 *w*<sup>1118</sup>; *P{XP}esc*<sup>d01514</sup> (BL#19163),  
182 *y*<sup>1</sup>  $w^*$ ; *pho*<sup>l81A/TM3</sup>, *Ser*<sup>1</sup> *y*<sup>+</sup> (BL#24164),

183 *red<sup>1</sup> e<sup>1</sup> ash2<sup>1</sup>/TM6B, Tb<sup>1</sup>* (BL#4584),  
184 *w<sup>1118</sup>; PBac{WH}Utx<sup>f01321</sup>/CyO* (BL#18425),  
185 *w<sup>\*</sup>; ash1<sup>22</sup> P{FRT(w<sup>hs</sup>)}2A/TM6C, Sb<sup>1</sup> Tb<sup>1</sup>* (BL#24161),  
186 *w<sup>1118</sup>; E(bx)<sup>Nurf301-3</sup>/TM3, P{ActGFP}JMR2, Ser<sup>1</sup>* (BL#9687),  
187 *y<sup>1</sup> w<sup>67c23</sup>; P{lacW}Nurf-38<sup>k16102</sup>/CyO* (BL#12206),  
188 *Mi-2<sup>4</sup> red<sup>1</sup> e<sup>4</sup>/TM6B, Sb<sup>1</sup> Tb<sup>1</sup> ca<sup>1</sup>* (BL#26170),  
189 *mor* RNAi (VDRC#6969),  
190 *psq<sup>E39</sup>/CyO; ry<sup>506</sup>* (BL#7321),  
191 *Rbf<sup>f4</sup> w<sup>1118</sup>/FM7c* (BL#7435),  
192 *w<sup>1118</sup> P{EP}Dsp1<sup>EP355</sup>* (BL#17270),  
193 *cn<sup>1</sup> grh<sup>IM</sup> bw<sup>1</sup>/SM6a* (BL#3270),  
194 *y<sup>1</sup> w<sup>67c23</sup>; P{lacW}lolal<sup>k02512</sup>/CyO* (BL#10515),  
195 *w<sup>\*</sup>; P{neoFRT}42D Pcl<sup>5</sup>/CyO* (BL#24157),  
196 *w<sup>\*</sup>; HDAC1<sup>def24</sup> P{FRT(w<sup>hs</sup>)}2A P{neoFRT}82B/TM6B, Tb<sup>1</sup>* (BL#32239),  
197 *w<sup>1118</sup>; Sirt1<sup>2A-7-11</sup>* (BL#8838),  
198 *Eip74EF<sup>v4</sup> vtd<sup>4</sup>/TM3, st<sup>24</sup> Sb<sup>1</sup>* (BL#5050),  
199 *sc<sup>1</sup> z<sup>1</sup> w<sup>is</sup>; Su(z)2<sup>1.b7</sup>/CyO* (BL#5572),  
200 *P{PZ}gpp<sup>03342</sup> ry<sup>506</sup>/TM3, ry<sup>RK</sup> Sb<sup>1</sup> Ser<sup>1</sup>* (BL#11585),  
201 *y<sup>1</sup> w<sup>1118</sup>; P{lacW}mod(mdg4)<sup>L3101</sup>/TM3, Ser<sup>1</sup>* (BL#10312),  
202 *w<sup>1118</sup>; PBac{RB}su(Hw)<sup>e04061</sup>/TM6B, Tb<sup>1</sup>* (BL#18224),  
203 *cn<sup>1</sup> P{PZ}lid<sup>10424</sup>/CyO; ry<sup>506</sup>* (BL#12367),  
204 *Asx<sup>XF23</sup>/CyO* (BL#6041),  
205 *y<sup>1</sup> w<sup>1</sup>; P{neoFRT}40A P{FRT(w<sup>hs</sup>)}G13 cn<sup>1</sup> PBac{SAsto-*  
206 *pDsRed}dom<sup>LL05537</sup> bw<sup>1</sup>/CyO, bw<sup>1</sup>* (BL#34496),  
207 *cn<sup>1</sup> E(Pc)<sup>1</sup> bw<sup>1</sup>/SM5* (BL#3056),  
208 *kis<sup>1</sup> cn<sup>1</sup> bw<sup>1</sup> sp<sup>1</sup>/SM6a* (BL#431),



209 *kto<sup>1</sup> ca<sup>1</sup>/TM6B, Tb<sup>1</sup>* (BL#3618),  
210 *skd<sup>2</sup>/TM6C, cu<sup>1</sup> Sb<sup>1</sup> ca<sup>1</sup>* (BL#5047).

211

## 212 **Genetic screen**

213 Mutants or RNAi lines were crossed to *w<sup>1118</sup>*; *rnGAL4, UAS-rpr, tub-*  
214 *GAL80<sup>ts</sup>/TM6B, tubGAL80* flies. Controls were *w<sup>1118</sup>* or the appropriate RNAi  
215 background line. Embryos were collected at room temperature on grape plates  
216 for 4 hours in the dark, then kept at 18°C. Larvae were picked at 2 days after egg  
217 lay into standard Bloomington cornmeal media and kept at 18°C, 50 larvae in  
218 each vial, 3 vials per genotype per replicate. On day 7, tissue ablation was in-  
219 duced by a placing the vials in a 30°C circulating water bath for 24 hours. Then  
220 ablation was stopped by placing the vials in ice water for 60 seconds and return-  
221 ing them to 18°C for regeneration. The regeneration index was calculated by  
222 summing the product of approximate wing size (0%, 25%, 50%, 75% and 100%)  
223 and the corresponding percentage of wings for each wing size. The  $\Delta$  Index was  
224 calculated by subtracting the regeneration index of the control from the regenera-  
225 tion index of the mutant or RNAi line.

226

227 To observe and quantify the patterning features and absolute wing size, adult  
228 wings that were 75% size or greater were mounted in Gary's Magic Mount (Can-  
229 ada balsam (Sigma) dissolved in methyl salicylate (Sigma)). The mounted adult  
230 wings were imaged with an Olympus SZX10 microscope using an Olympus  
231 DP21 camera, with the Olympus CellSens Dimension software. Wings were  
232 measured using ImageJ.

233

## 234 **Immunostaining**

235 Immunostaining was carried out as previously described (Smith-Bolton et al.,  
236 2009). Primary antibodies used in this study were rabbit anti-Myc (1:500; Santa  
237 Cruz Biotechnology), mouse anti-Nubbin (1:250; gift from Steve Cohen) (Ng et  
238 al., 1996), mouse anti-engrailed/inverted (1:3; Developmental Studies Hybrid-  
239 oma Bank (DSHB)) (Patel et al., 1989), mouse anti-Patched (1:50; DSHB)  
240 (Capdevila et al., 1994), mouse anti-Achaete (1:10; DSHB) (Skeath and Carroll,  
241 1992), rabbit anti-PH3 (1:500; Millipore), mouse anti-Osa (1:1; DSHB) (Treisman  
242 et al., 1997), rat anti-Ci (1:10; DSHB) (Motzny and Holmgren, 1995), rabbit anti-  
243 Dcp1 (1:250; Cell Signaling), mouse anti- $\beta$ gal (1:100; DSHB), rabbit anti-phos-  
244 pho-Mad (1:100; Cell Signaling), mouse anti-Mmp1 (1:10 of 1:1:1 mixture of  
245 monoclonal antibodies 3B8D12, 5H7B11, and 3A6B4, DSHB)(Page-McCaw et  
246 al., 2003). The Developmental Studies Hybridoma Bank (DSHB) was created by  
247 the NICHD of the NIH and is maintained at the University of Iowa, Department of  
248 Biology, Iowa City, IA 52242. Secondary antibodies used in this study were  
249 AlexaFluor secondary antibodies (Molecular Probes) (1:1000). TO-PRO-3 iodide  
250 (Molecular Probes) was used to detect DNA at 1:500.

251

252 Confocal images were collected with a Zeiss LSM700 Confocal Microscope using  
253 ZEN software (Zeiss). Images were processed with ImageJ (NIH) and Photoshop  
254 (Adobe). Average fluorescence intensity was measured by ImageJ. Quantifica-  
255 tion of fluorescence intensity and phospho-histone H3 positive cells was re-  
256 stricted to the wing pouch, as marked by anti-Nubbin immunostaining or morphol-  
257 ogy. The area of the regenerating wing primordium was quantified by measuring  
258 the anti-Nubbin immunostained area in ImageJ.

259

260 **Quantitative RT-PCR**

261 qPCR was conducted as previously described (Skinner et al., 2015). Each inde-  
262 pendent sample consisted of 50 wing discs. 3 biological replicates were collected  
263 for each genotype and time point. Expression levels were normalized to the con-  
264 trol *gapdh2*. The fold changes compared to the *w<sup>1118</sup>* undamaged wing discs are  
265 shown. Primers used in the study were:

266 *GAPDH2* (Forward: 5'-GTGAAGCTGATCTCTTGGTACGAC-3';

267 Reverse: 5'-CCGCGCCCTAATCTTTAACTTTTAC-3'),

268 *ilp8* (Qiagen QT00510552),

269 *mmp1* (Forward: 5'-TCGGCTGCAAGAACACGCCC-3';

270 Reverse: 5'-CGCCCACGGCTGCGTCAAAG-3'),

271 *moira* (Forward: 5'-GATGAGGTGCCCGCTACAAT-3';

272 Reverse: 5'-CTGCTGCGGTTTCGTCTTTT-3'),

273 *brm* (Forward: 5'-GCACCACCAGGGGATGATTT-3';

274 Reverse: 5'-TTGTGTGGGTGCATTGGGT-3'),

275 *Bap60* (Forward: 5'-AGACGAGGGATTTGAAGCTGA-3';

276 Reverse: 5'-AGGTCTCTTGACGGTGGACT-3')

277 *myc* (Forward: 5'-CGATCGCAGACGACAGATAA-3';

278 Reverse: 5'-GGGCGGTATTAAATGGACCT-3')

279

## 280 **Pupariation timing experiments**

281 To quantify the pupariation rates, pupal cases on the side of each vial were  
282 counted at 24-hour intervals starting from the end of tissue ablation until no new  
283 pupal cases formed. Three independent biological replicates, which consisted of  
284 3 vials each with 50 animals per vial, were performed for each experiment. The  
285 median day is the day on which  $\geq 50\%$  of the animals had pupariated.

286

## 287 **Data Availability**

288 All relevant data are available at [databank.illinois.edu](http://databank.illinois.edu).XXXXXXXXX and upon re-  
289 quest.

290

291

## 292 **Results**

### 293 **A genetic screen of chromatin modifier mutants and RNAi lines**

294 To identify regeneration genes among *Drosophila* chromatin regulators, we con-  
295 ducted a genetic screen similar to our previously reported unbiased genetic  
296 screen for genes that regulate wing imaginal disc regeneration (Brock et al.,  
297 2017)(Fig. 1A). To induce tissue ablation, *rotund-GAL4* drove the expression of  
298 the pro-apoptotic gene *UAS-reaper* in the imaginal wing pouch, and *tubulin-*  
299 *GAL80<sup>ts</sup>* provided temporal control, enabling us to turn ablation on and off by var-  
300 ying the temperature (Smith-Bolton et al., 2009). The ablation was carried out for  
301 24 hours during the early third instar. We characterized the quality of regenera-  
302 tion by assessing the adult wing size semi-quantitatively and 1) recording the  
303 numbers of wings that were 0%, 25%, 50%, 75% or 100% the length of a normal  
304 adult wing (Fig. 1A,B), and 2) identifying patterning defects by scoring ectopic or  
305 missing features. This semi-quantitative evaluation method enabled a quick  
306 screen, at a rate of 6 genotypes per week including around 1400 adult wings,  
307 and identification of both enhancers and suppressors of regeneration (Fig. 1B-E).  
308 While control animals regenerated to varying degrees depending on the extent  
309 they delayed metamorphosis in response to damage (Khan et al., 2017; Smith-  
310 Bolton et al., 2009) as well as seasonal differences in humidity and food quality  
311 (Skinner et al., 2015), the differences between the regenerative capacity of mu-  
312 tants and controls were consistent (Brock et al., 2017; Khan et al., 2017; Smith-  
313 Bolton et al., 2009).

314

315 Using this system, we screened mutants and RNAi lines affecting chromatin reg-  
316 ulators (Table S1, Fig. 1C, Fig. S1A). For each line, we calculated the  $\Delta$  regener-  
317 ation index, which is the difference between the regeneration indices of the line  
318 being tested and the control tested simultaneously (see materials and methods  
319 for regeneration index calculation). We set a cutoff  $\Delta$  index of 10%, over which  
320 we considered the regenerative capacity to be affected. Seventy-eight percent of  
321 the mutants and RNAi lines tested had a change in regeneration index of 10% or  
322 more compared to controls (Table S1, Fig. 1C, Fig. S1A), consistent with the idea  
323 that changes in chromatin structure are required for the damaged tissue to exe-  
324 cute the regeneration program. Twenty-two percent of the mutants and RNAi  
325 lines failed to meet our cutoff and were not pursued further (Table S1, Fig. 1C).  
326 Strikingly, 41% of the tested lines, such as *pho1<sup>B1A</sup>/+*, which affects the PhoRC  
327 complex, had larger adult wings after ablation and regeneration compared to  
328 control *w<sup>1118</sup>* animals that had also regenerated (Fig. 1D), indicating enhanced  
329 regeneration, although none were larger than a normal-sized wing. By contrast,  
330 25% of the tested lines, such as *E(bx)<sup>nurf301-3</sup>/+*, which affects the NURF complex,  
331 had smaller wings (Fig. 1E), indicating worse regeneration. Unexpectedly, muta-  
332 tions that affected the same complex did not have consistent phenotypes (Table  
333 S1), suggesting that chromatin modification and remodeling likely regulate a deli-  
334 cate balance of genes that promote and constrain regeneration. Indeed, tran-  
335 scriptional profiling has identified a subset of genes that are upregulated after  
336 wing disc ablation (Khan et al., 2017), some of which promote regeneration, and  
337 some of which constrain regeneration, indicating that gene regulation after tissue  
338 damage is not as simple as turning on genes that promote regeneration and turn-  
339 ing off genes that inhibit regeneration.

340

341 **The SWI/SNF PBAP and BAP complexes have opposite phenotypes.**

342 To clarify the roles of one type of chromatin-regulating complex in regeneration,  
343 we focused on the SWI/SNF chromatin-remodeling complexes (Fig. 2A). As  
344 shown in Table S1, different components of the SWI/SNF complexes showed dif-  
345 ferent phenotypes after ablation and regeneration of the wing pouches. Animals  
346 heterozygous mutant for the PBAP-specific components *Bap170* (*Bap170<sup>Δ135/+</sup>*)  
347 and Polybromo (*polybromo<sup>Δ86/+</sup>*) had adult wings that were smaller after disc re-  
348 generation than *w<sup>1118</sup>* adult wings after disc regeneration (Fig. 2B,C), suggesting  
349 that the PBAP complex is required for ablated wing discs to regrow. To confirm  
350 these semiquantitative results, we mounted adult wings and measured absolute  
351 wing sizes (N≥100 wings for each genotype). The reduced regeneration of  
352 *Bap170<sup>Δ135/+</sup>* wing discs was confirmed by measurement of the adult wings (Fig.  
353 2E). By contrast, animals heterozygous mutant for the BAP-specific component  
354 *Osa* (*osa<sup>308/+</sup>*) had larger adult wings after disc regeneration compared to *w<sup>1118</sup>*  
355 adult wings after disc regeneration (Fig. 2D), suggesting that impairment of the  
356 BAP complex deregulates growth after tissue damage. Measurement of the adult  
357 wings of *osa<sup>308/+</sup>* animals after disc regeneration confirmed the enhanced regen-  
358 eration (Fig. 2F).

359

360 Interestingly, the *osa<sup>308/+</sup>* adult wings also showed severe patterning defects af-  
361 ter damage and regeneration of the disc (Fig. 2G-I). Specifically, the posterior  
362 compartment of the *osa<sup>308/+</sup>* wings had anterior features after wing pouch abla-  
363 tion, but had normal wings when no tissue damage was induced (Fig. S1B). To  
364 quantify the extent of the posterior-to-anterior (P-to-A) transformations, we quan-  
365 tified the number of anterior features in the posterior of each wing, including

366 socketed bristles and ectopic veins on the posterior margin, an ectopic anterior  
367 crossvein (ACV), costal bristles on the alula, and an altered shape that has a nar-  
368 rower proximal and wider distal P compartment (Schuster and Smith-Bolton,  
369 2015) (Fig. 2I). While *w<sup>1118</sup>* adult wings that had regenerated as discs had a low  
370 level of P-to-A transformations, 75% of the *osa<sup>308/+</sup>* wings had P-to-A transfor-  
371 mations, and 83% of these transformed wings had 4 or 5 anterior markers in the  
372 posterior of the wing. Thus, Osa is required to preserve posterior cell fate during  
373 regeneration, suggesting that the BAP complex regulates cell fate after damage.  
374

### 375 **Reducing the core SWI/SNF components to varying levels produces either** 376 **the BAP or PBAP phenotype**

377 Because mutants of the BAP or PBAP complex-specific components showed dis-  
378 tinct phenotypes, we also screened mutants of the core components for regener-  
379 ation phenotypes. Interestingly, mutants or RNAi lines that reduced levels of the  
380 core components were split between the two phenotypes. For example, *brm<sup>2/+</sup>*  
381 discs and discs expressing a *Bap111* RNAi construct regenerated poorly, result-  
382 ing in small wings (Fig. 3A,B), while *Bap55<sup>LL05955/+</sup>* discs, *mor<sup>1/+</sup>* discs, and discs  
383 expressing a *Bap60* RNAi construct regenerated to produce larger wings overall  
384 that showed P-to-A transformations (Table S1, Fig. 3C-G, Fig. S1A).

385  
386 Given that the SWI/SNF complexes require the function of the scaffold Mor and  
387 the ATPase Brm (Mashtalir et al., 2018; Moshkin et al., 2007), it was surprising  
388 that reduction of Mor showed the BAP phenotype while reduction of Brm showed  
389 the PBAP phenotype. However, it is likely that some of the mutants and RNAi  
390 lines caused stronger loss of function than others. A stronger reduction in func-  
391 tion would result in malfunction of both BAP and PBAP, and show the reduced

392 regeneration phenotype, masking any patterning defects. By contrast, a weaker  
393 reduction in function could mainly affect the BAP complex. For example, *Bap60*  
394 RNAi, which caused patterning defects after wing disc regeneration, only induced  
395 a moderate reduction in mRNA levels, suggesting that it causes a weak loss of  
396 function (Fig S1C). Although it is unclear why a weaker reduction of function  
397 would mainly affect the BAP complex, it is possible that the BAP complex is less  
398 abundant than the PBAP complex, such that a slight reduction in a core compo-  
399 nent would have a greater effect on the amount of BAP in the tissue. Therefore,  
400 we hypothesized that stronger or weaker loss of function of the same core com-  
401 plex component might show different phenotypes.

402

403 To test this hypothesis, we used a strong loss-of-function *mor* mutant, *mor<sup>11</sup>* (gift  
404 from J. Kennison, Fig. S1D), and two hypomorphic *mor* mutants *mor<sup>1</sup>* and *mor<sup>2</sup>*  
405 (Kennison and Tamkun, 1988). Indeed, *mor<sup>11</sup>/+* undamaged wing discs had sig-  
406 nificantly less *mor* transcript than *mor<sup>1</sup>/+* or control undamaged wing discs (Fig.  
407 3H). Interestingly, *mor<sup>11</sup>/+* animals showed the poor regeneration phenotype sim-  
408 ilar to the PBAP complex-specific *Bap170<sup>Δ135</sup>/+* mutants (Fig. 3I), while *mor<sup>1</sup>/+*  
409 and *mor<sup>2</sup>/+* showed the enhanced regeneration phenotype and the P-to-A trans-  
410 formation phenotype similar to the BAP complex-specific *osa<sup>308</sup>/+* mutants (Fig.  
411 3E,J, S1Table). To confirm these findings we also used an amorphic allele of *brm*  
412 and an RNAi line that targets *brm* to reduce the levels of the core component  
413 *brm*. *brm<sup>2</sup>* was generated through ethyl methanesulfonate mutagenesis and  
414 causes a loss of Brm protein (Elfring et al., 1998; Kennison and Tamkun, 1988).  
415 The *brm* RNAi causes a partial reduction in transcript, as *rn>brmRNAi* undam-  
416 aged wing discs had less *brm* transcript than control undamaged wing discs (Fig.  
417 S1E). *brm<sup>2</sup>/+* animals showed the small wing phenotype after disc damage,



418 indicating poor regeneration (Fig. 3A). By contrast, knockdown of *brm* by ex-  
419 pressing the *brm* RNAi construct during tissue ablation induced larger wings and  
420 P-to-A transformations (Fig. 3K,L). Thus, slight reduction of the core SWI/SNF  
421 components, through *mor*<sup>1</sup>, *brm* RNAi, or *Bap60* RNAi, produced the BAP pheno-  
422 type, whereas stronger reduction of the core components, through *mor*<sup>11</sup>, pro-  
423 duced the PBAP phenotype, suggesting that it is easier to compromise BAP  
424 function than to compromise PBAP function. If it is easier to compromise BAP  
425 function because there is less BAP complex in regenerating wing disc cells, over-  
426 expression of the BAP-specific component *Osa* would lead to an increase in the  
427 amount of BAP complex and rescue the *brm* RNAi phenotype. Indeed, overex-  
428 pression of *osa* in regenerating tissue rescued the enhanced wing size and P-to-  
429 A transformations induced by *brm* RNAi (Fig. 3M,N).

430

### 431 **The PBAP complex is required for Myc upregulation and cell proliferation** 432 **during regrowth**

433 To identify when the defect in regrowth occurs in PBAP complex mutants, we  
434 measured the regenerating wing pouch using expression of the pouch marker  
435 *nubbin* in *w*<sup>1118</sup> controls, *Bap170*<sup>Δ135/+</sup> and *brm*<sup>2/+</sup> mutants, as well as in the  
436 *osa*<sup>308/+</sup> BAP mutant for comparison. The regenerating wing pouches of  
437 *Bap170*<sup>Δ135/+</sup> mutant animals were not different in size compared to *w*<sup>1118</sup> animals  
438 at 0, 12, or 24 hours after tissue damage (R0, R12 or R24). However, the  
439 *Bap170*<sup>Δ135/+</sup> regenerating wing pouches were smaller than *w*<sup>1118</sup> by 36 hours af-  
440 ter tissue damage (R36), shortly before the *Bap170*<sup>Δ135/+</sup> mutant animals pupari-  
441 ated and entered metamorphosis (Fig. 4A-C). *brm*<sup>2/+</sup> mutant animals also had  
442 smaller regenerating wing pouches by R24 (Fig. S2A-C). By contrast, the regen-  
443 erating *osa*<sup>308/+</sup> wing pouches regrew at the same rate as controls (Fig. S2D-H).

444

445 To determine whether the *Bap170<sup>Δ135/+</sup>* mutant animals had a slower rate of pro-  
446 liferation during regeneration, we quantified the number of mitotic cells by im-  
447 munostaining for phospho-histone H3 (PH3) in the regenerating wing pouch. A  
448 35% decrease in the number of PH3-positive cells was observed in *Bap170<sup>Δ135/+</sup>*  
449 mutants (Fig. 4D-F, Fig. S2I). While smaller adult wings could also be caused by  
450 increased cell death in the regenerating tissue, we did not find an increase in cell  
451 death in *Bap170<sup>Δ135/+</sup>* regenerating wing discs as marked by immunostaining for  
452 cleaved caspase Dcp1 (Fig. S2J,K).

453

454 To identify why proliferation was reduced in *Bap170<sup>Δ135/+</sup>* mutants, we examined  
455 levels of Myc, an important growth regulator that is upregulated during *Drosoph-*  
456 *ila* wing disc regeneration (Smith-Bolton et al., 2009). In mammals, *c-myc* is a di-  
457 rect target of the SWI/SNF BAF complex, which is similar to *Drosophila* BAP  
458 (Nagl et al., 2006), but a role for the PBAP complex in regulating the *Drosophila*  
459 *Myc* gene has not been established. Myc protein levels were significantly re-  
460 duced in *Bap170<sup>Δ135/+</sup>* and *brm<sup>2/+</sup>* regenerating wing pouches compared to wild-  
461 type regenerating wing pouches (Fig. 4G-I and Fig. S3A-D). Myc transcriptional  
462 levels were also significantly lower in *Bap170<sup>Δ135/+</sup>* regenerating wing discs com-  
463 pared to wild-type regenerating discs (Fig. 4J). By contrast, there was no change  
464 in Myc levels in *osa<sup>308/+</sup>* mutants (Fig. S3E-G), indicating that PBAP, but not  
465 BAP, is required for upregulation of Myc after tissue damage. To determine the  
466 extent to which reduction of Myc expression was responsible for the poor regen-  
467 eration phenotype in BAP complex mutants, we overexpressed Myc in the  
468 *Bap170<sup>Δ135/+</sup>* background during regeneration. Indeed, the *Bap170<sup>Δ135/+</sup>, UAS-*  
469 *Myc/+* animals regenerated similar to the *w<sup>1118</sup>* controls and significantly better

470 than *Bap170<sup>Δ135/+</sup>* animals, demonstrating partial rescue of the poor regeneration  
471 phenotype (Fig. 4K and Fig. S3H).

472

### 473 **The PBAP complex is required for the delay in pupariation induced by tis-** 474 **sue damage**

475 Damaged imaginal discs delay pupariation by expressing the peptide ILP8, which  
476 delays the production of ecdysone and onset of metamorphosis, providing more  
477 time for damaged tissue to regenerate (Colombani et al., 2012; Garelli et al.,  
478 2012). To determine whether the SWI/SNF complexes regulate the timing of met-  
479 amorphosis, we quantified the pupariation rate in *w<sup>1118</sup>* and *Bap170<sup>Δ135/+</sup>* regen-  
480 erating animals, and identified the day on which 50% of the larvae had pupari-  
481 ated. Without tissue damage, *Bap170<sup>Δ135/+</sup>* mutants pupariated slightly later than  
482 *w<sup>1118</sup>* animals (Fig. 4L and Fig. S4A), but the difference is not significant. How-  
483 ever, after wing disc damage, more than half of the *Bap170<sup>Δ135/+</sup>* mutant animals  
484 had pupariated by 2 days after damage, whereas more than half of the *w<sup>1118</sup>* ani-  
485 mals had not pupariated until 3 days after damage, giving the mutants 1/3 less  
486 time to regenerate (Fig. 4M and Fig. S4B). To uncover why *Bap170<sup>Δ135/+</sup>* animals  
487 had less regeneration time, we quantified *ilp8* transcript levels. Indeed,  
488 *Bap170<sup>Δ135/+</sup>* animals had about 50% less *ilp8* mRNA (Fig. 4N), suggesting that  
489 the PBAP complex is required for *ilp8* expression.

490

### 491 **The PBAP complex regulates expression of JNK signaling targets**

492 SWI/SNF complexes can be recruited by transcription factors to act as co-activa-  
493 tors of gene expression (Becker and Workman, 2013). Regenerative growth and  
494 the pupariation delay are regulated by JNK signaling (Bergantinos et al., 2010;  
495 Bosch et al., 2008; Colombani et al., 2012; Garelli et al., 2012; Skinner et al.,

496 2015). Thus, it is possible that PBAP is recruited to JNK signaling targets like *ilp8*  
497 by the AP-1 transcription factor, which acts downstream of JNK (Perkins et al.,  
498 1988), and that PBAP is required for full activation of these targets. To determine  
499 whether *Bap170* is required for JNK-dependent transcription, we examined the  
500 activity of the TRE-Red reporter, which is comprised of four AP-1 binding sites  
501 (TREs) driving the expression of a DsRed.T4 reporter gene (Chatterjee and  
502 Bohmann, 2012) in *w<sup>1118</sup>* and *Bap170<sup>Δ135/+</sup>* regenerating wing discs. The TRE-  
503 Red intensity was significantly decreased in the *Bap170<sup>Δ135/+</sup>* regenerating tissue  
504 compared to the *w<sup>1118</sup>* regenerating tissue (Fig. 4O-R), indicating that PBAP is re-  
505 quired for full activation of this AP-1 transcriptional activity reporter, similar to its  
506 requirement for expression of *ilp8*. Furthermore, expression of the JNK signaling  
507 target *mmp1* was significantly reduced in *Bap170<sup>Δ135/+</sup>* regenerating wing discs  
508 at both the mRNA and protein levels (Fig. 4S and Fig. S4C-E). Thus, the PBAP  
509 complex plays a crucial role in activation of JNK signaling targets.

510

### 511 **The BAP complex maintains posterior cell fate during regeneration**

512 After damage and regeneration of the disc, adult wings of *osa<sup>308/+</sup>*,  
513 *Bap55<sup>LL05955/+</sup>*, *mor<sup>1/+</sup>*, and *mor<sup>2/+</sup>* discs, as well as discs expressing a *brm* RNAi  
514 construct or a *Bap60* RNAi construct, had anterior bristles and veins in the poste-  
515 rior compartment (Fig. 3C-G,K), but not after normal development (Fig. S1A,  
516 S1F-H). To identify when the P-to-A transformations occurred, we examined the  
517 expression of anterior- and posterior-specific genes during tissue regeneration.  
518 *engrailed (en)* is essential for posterior cell fate both in development and regen-  
519 eration (Kornberg et al., 1985; Schuster and Smith-Bolton, 2015). To assess abil-  
520 ity to maintain posterior cell fate, regenerating wing discs were dissected at dif-  
521 ferent times during recovery (R) and immunostained for the posterior selector

522 gene *en*. At 72 hours after damage (R72), in *osa*<sup>308/+</sup> regenerating discs, *en* was  
523 expressed in some of the posterior compartment, but lost in patches (Fig. 5A-C).  
524 In addition, the proneural protein Acheate (Ac), which is expressed in sensory or-  
525 gan precursors in the anterior of wing discs (Skeath and Carroll, 1991), was ec-  
526 topically expressed in the posterior (Fig. 5D-F) marking precursors to the ectopic  
527 socketed bristles found in the posterior of the adult wings. The anterior genes *cu-*  
528 *bitus interruptus* (*ci*) (Eaton and Kornberg, 1990) and *patched* (*ptc*) (Phillips et  
529 al., 1990) were also ectopically expressed in the posterior of the *osa*<sup>308/+</sup> R72 re-  
530 generating wing discs (Fig. 6A-C). The ectopic expression of these anterior  
531 genes was not observed at R48, suggesting that the P-to-A fate transformations  
532 happened late during regeneration (Fig. S4F,G). Similarly, at R72, 80% of the  
533 *brm* RNAi wing discs had ectopic expression of the anterior genes *ptc* and *ci* in  
534 the posterior of the discs, while no expression of *ptc* or *ci* was observed in the  
535 posterior of control R72 discs (Fig. 6D,E).

536

537 We previously showed that in *Drosophila* wing disc regeneration, elevated JNK  
538 increases expression of *en*, leading to PRC2-mediated silencing of the *en* locus  
539 in patches, and transformation of the *en*-silenced cells to anterior fate, and that  
540 Taranis prevents this misregulation of *en* and resulting P-to-A cell fate transfor-  
541 mations (Schuster and Smith-Bolton, 2015). Thus, we wondered whether the  
542 BAP complex preserved *en* expression and posterior fate by reducing JNK sig-  
543 naling, or regulating *tara* expression, or working in parallel to Tara during the  
544 later stages of regeneration.

545

546 **The BAP complex does not regulate JNK signaling**

547 To determine whether the BAP complex regulates JNK signaling, we examined  
548 the JNK reporter TRE-Red in *osa*<sup>308/+</sup> and *w*<sup>1118</sup> regenerating wing discs. In con-  
549 trast to *Bap170*<sup>Δ135/+</sup> mutants (Fig. 4O-R), TRE-Red intensity was not different  
550 between *osa*<sup>308/+</sup> and *w*<sup>1118</sup> regenerating tissue (Fig. 7A-C). Thus, the BAP com-  
551 plex acts to protect posterior cell fate downstream of or in parallel to JNK signal-  
552 ing.

553

### 554 **The BAP complex functions in parallel to Taranis to preserve cell fate**

555 Because *tara* is regulated transcriptionally after tissue damage (Schuster and  
556 Smith-Bolton, 2015), we examined whether the BAP complex is required for *tara*  
557 upregulation in the regenerating tissue. Using a *tara-lacZ* enhancer trap, we as-  
558 sessed expression in *Bap55*<sup>LL05955/+</sup> regenerating wing discs, which had the  
559 same P-to-A transformations as the *osa*<sup>308/+</sup> regenerating discs. No change in  
560 *tara-lacZ* expression was identified in the regenerating wing pouches, (Fig. 7D-  
561 G), indicating that the damage-dependent *tara* expression was not downstream  
562 of BAP activity.

563

564 To determine whether Tara can suppress the P-to-A transformations induced by  
565 the reduction of BAP, we overexpressed Tara using *UAS-tara* under control of *rn-*  
566 *Gal4* in the *osa*<sup>308/+</sup> mutant animals, generating elevated Tara levels in the *rn-ex-*  
567 pressing cells that survived the tissue ablation. Indeed, the P-to-A transformation  
568 phenotype in *osa*<sup>308/+</sup> mutant animals was rescued by Tara overexpression (Fig.  
569 7H-K). To rule out the possibility that Tara regulates *osa* expression, we quanti-  
570 fied Osa immunostaining in *tara*<sup>1/+</sup> mutant regenerating tissue. Osa protein levels  
571 did not change during regeneration, and were unchanged in *tara*<sup>1/+</sup> mutant re-  
572 generating discs (Fig. S4H-M). Taken together, these data indicate that the BAP

573 complex likely functions in parallel to Tara to constrain *en* expression, preventing  
574 auto-regulation and silencing of *en*, thereby protecting cell fate from changes in-  
575 duced by JNK signaling during regeneration.

576

577 **The enhanced growth in BAP mutants is caused by ectopic AP boundaries.**

578 The increased wing size after disc regeneration in *tara*/+ animals was due to loss  
579 of *en* in patches of cells, which generated aberrant juxtaposition of anterior and  
580 posterior tissue within the posterior compartment. These ectopic AP boundaries  
581 established ectopic Dpp morphogen gradients (Schuster and Smith-Bolton,  
582 2015), which can stimulate extra growth in the posterior compartment (Tanimoto  
583 et al., 2000). To determine whether the *osa*/+ regenerating discs also had ectopic  
584 AP boundaries and ectopic morphogen gradients, we immunostained for Ptc to  
585 mark AP boundaries and phospho-Smad to visualize gradients of Dpp signaling.  
586 Indeed, Ectopic regions of Ptc expression were surrounded by ectopic pSmad  
587 gradients in *osa*<sup>308</sup>/+ regenerating discs (Fig. 8A-C). Thus, the enhanced regen-  
588 eration in *osa*<sup>308</sup>/+ and other SWI/SNF mutant animals was likely a secondary re-  
589 sult of the patterning defect. Furthermore, pupariation occurred later in *osa*<sup>308</sup>/+  
590 regenerating animals compared to *w*<sup>1118</sup> regenerating animals (Fig. S4N,O),  
591 which provided more time for regeneration in the mutants. Such a delay in pupar-  
592 iation can be caused by aberrant proliferation (Colombani et al., 2012; Garelli et  
593 al., 2012) in addition to tissue damage, and the combination of the two likely led  
594 to the increase in delay in metamorphosis seen specifically in mutants with P-to-  
595 A transformations.

596

597 **Discussion**

598 To address the question of how regeneration genes are regulated in response to  
599 tissue damage, we screened a collection of mutants and RNAi lines that affect a  
600 significant number of the chromatin regulators in *Drosophila*. Most of these mu-  
601 tants had regeneration phenotypes, confirming that these genes are important for  
602 both promoting and constraining regeneration and likely facilitate the shift from  
603 the normal developmental program to the regeneration program, and back again.  
604 The variation in regeneration phenotypes among different chromatin regulators  
605 and among components of the same multi-unit complexes supports our previous  
606 finding that damage activates expression of genes that both promote and con-  
607 strain regeneration (Khan et al., 2017). Such regulators of regeneration may be  
608 differentially affected by distinct mutations that affect the same chromatin-modify-  
609 ing complexes, resulting in different phenotypes.

610

611 We have demonstrated that both *Drosophila* SWI/SNF complexes play essential  
612 but distinct roles during epithelial regeneration, controlling multiple aspects of the  
613 process, including growth, developmental timing, and cell fate (Fig. 8D). Further-  
614 more, our work has identified multiple likely targets, including *mmp1*, *myc*, *ilp8*,  
615 and *en*. Indeed, analysis of data from a recent study that identified regions of the  
616 genome that transition to open chromatin after imaginal disc damage showed  
617 such damage-responsive regions near *Myc*, *mmp1*, and *ilp8* (Vizcaya-Molina et  
618 al., 2018). While previous work has suggested that chromatin modifiers can regu-  
619 late regeneration (Blanco et al., 2010; Fukuda et al., 2012; Jin et al., 2013; Jin et  
620 al., 2015; Pfefferli et al., 2014; Scimone et al., 2010; Skinner et al., 2015; Stewart  
621 et al., 2009; Sun et al., 2016; Tseng et al., 2011; Wang et al., 2008; Xiong et al.,  
622 2013), and that the chromatin near *Drosophila* regeneration genes is modified af-  
623 ter damage (Harris et al., 2016; Vizcaya-Molina et al., 2018), our results suggest



624 that these damage-responsive loci are not all coordinately regulated in the same  
625 manner. The SWI/SNF complexes target different subsets of genes, and it will  
626 not be surprising if different cofactors or transcription factors recruit different  
627 complexes to other subsets of regeneration genes.

628

629 Is the requirement for the SWI/SNF complexes for growth and conservation of  
630 cell fate in the wing disc specific to regeneration? In contrast to *tara*, which is re-  
631 quired for posterior wing fate only after damage and regeneration (Schuster and  
632 Smith-Bolton, 2015), loss of *mor* in homozygous clones during wing disc devel-  
633 opment caused loss of *en* expression in the posterior compartment (Brizuela and  
634 Kennison, 1997), although this result was interpreted to mean that *mor* promotes  
635 rather than constrains *en* expression, which is the opposite of our observations.  
636 Importantly, undamaged *mor* heterozygous mutant animals did not show pattern-  
637 ing defects (Fig. S1G,H), while damaged heterozygous mutant animals did (Fig.  
638 3E), indicating that regenerating tissue is more sensitive to reductions in  
639 SWI/SNF levels than normally developing tissue. Furthermore, *osa* is required for  
640 normal wing growth (Terriente-Félix and de Celis, 2009), but reduction of *osa* lev-  
641 els did not compromise growth during regeneration (Fig. 2D). Thus, while some  
642 functions of SWI/SNF during regeneration may be the same as during develop-  
643 ment, other functions of SWI/SNF are unique to regeneration.

644

645 SWI/SNF complexes help organisms respond rapidly to stressful conditions or  
646 changes in the environment. For example, SWI/SNF is recruited by the transcrip-  
647 tion factor DAF-16/FOXO to promote stress resistance in *Caenorhabditis elegans*  
648 (Riedel et al., 2013), and the *Drosophila* BAP complex is required for the activa-  
649 tion of target genes of the NF- $\kappa$ B signaling transcription factor Relish in immune

650 responses (Bonnay et al., 2014). Here we show that the *Drosophila* PBAP com-  
651 plex is similarly required after tissue damage for activation of target genes of the  
652 JNK signaling transcription factor AP-1 after tissue damage. Interestingly, the  
653 BAF60a subunit, a mammalian homolog of *Drosophila* BAP60, directly binds the  
654 AP-1 transcription factor and stimulates the DNA-binding activity of AP-1 (Ito et  
655 al., 2001), suggesting that this role may be conserved.

656

657 In summary, we have demonstrated that the two SWI/SNF complexes regulate  
658 different aspects of wing imaginal disc regeneration, implying that activation of  
659 the regeneration program is controlled by changes in chromatin, but that the  
660 mechanism of regulation is likely different for subsets of regeneration genes. Fu-  
661 ture identification of all genes targeted by BAP and PBAP after tissue damage,  
662 the factors that recruit these chromatin-remodeling complexes, and the changes  
663 they induce at these loci will deepen our understanding of how unexpected or  
664 stressful conditions lead to rapid activation of the appropriate genes.

665

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672

## 673 **Competing interests**

674 No competing interests declared.

675

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680

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908 **Fig 1. A genetic screen of chromatin regulators identified important regen-**  
909 **eration genes**

910 (A) Method for screening mutants or RNAi lines using a genetic ablation system.

911 Mutants or RNAi lines of genes involved in regulating chromatin were crossed to  
912 the ablation stock (*w<sup>1118</sup>; +; rn-GAL4, UAS-rpr, tubGAL80<sup>ts</sup>/TM6B, tubGAL80*).

913 Animals were kept at 18°C until 7 days after egg lay (AEL), when they were  
914 moved to 30°C to induce tissue ablation for 24 hours, then transferred back to  
915 18°C to enable recovery (R). The size of the regenerated adult wings was as-  
916 sessed semi-quantitatively by counting the number of wings that were approxi-  
917 mately 0%, 25%, 50%, 75% or 100% of the length of a control adult wing that  
918 had not undergone damage during the larval phase. The regenerating discs were  
919 also examined at different times denoted by hours after the beginning of recov-  
920 ery, such as R0, R24, R48 and R72.

921 (B) Conceptual model for the screen to identify mutants or RNAi lines showing  
922 enhanced (green) or reduced (purple) regeneration compared to control.

923 (C) Summary of the screen of chromatin regulators, showing percent of lines  
924 tested that had a regeneration phenotype, as well as percent of those with a phe-  
925 notype that regenerated better ( $\Delta$  Index  $\geq 10\%$ ) or worse ( $\Delta$  Index  $\leq -10\%$ ) com-  
926 pared to controls.

927 (D) Comparison of the size of adult wings after imaginal disc damage and regen-  
928 eration in *pho1<sup>B1A</sup>/+* and wild-type (*w<sup>1118</sup>*) animals. n = 64 wings (*pho1<sup>B1A</sup>/+*) and  
929 242 wings (*w<sup>1118</sup>*) from 3 independent experiments. Chi-square test  $p < 0.001$   
930 across all wing sizes. Error bars are s.e.m.

931 (E) Comparison of the size of adult wings after imaginal disc damage and regen-  
932 eration in *E(bx)<sup>nurf301-3</sup>/+* and wild-type (*w<sup>1118</sup>*) animals. n = 219 wings (*E(bx)<sup>nurf301-</sup>*  
933 *3/+) and 295 wings (*w<sup>1118</sup>*) from 3 independent experiments. Chi-square test  $p <$   
934 0.001 across all wing sizes. Error bars are s.e.m.*

935

936 **Fig 2. SWI/SNF components Bap170, Polybromo and Osa are required for**  
937 **regeneration**

938 (A) Schematics of the two *Drosophila* SWI/SNF chromatin-remodeling com-  
939 plexes: BAP and PBAP, drawn based on complex organization determined in  
940 (Mashtalir et al., 2018).

941 (B) Comparison of the size of adult wings after imaginal disc damage and regen-  
942 eration in *Bap170<sup>Δ135</sup>/+* and wild-type (*w<sup>1118</sup>*) animals. n = 190 wings  
943 (*Bap170<sup>Δ135</sup>/+*) and 406 wings (*w<sup>1118</sup>*) from 3 independent experiments. Chi-  
944 square test  $p < 0.001$  across all wing sizes.

945 (C) Comparison of the size of adult wings after imaginal disc damage and regen-  
946 eration in *polybromo<sup>Δ86</sup>/+* and wild-type (*w<sup>1118</sup>*) animals. n = 180 wings  
947 (*polybromo<sup>Δ86</sup>/+*) and 396 wings (*w<sup>1118</sup>*) from 3 independent experiments. Chi-  
948 square test  $p < 0.001$  across all wing sizes.

949 (D) Comparison of the size of adult wings after imaginal disc damage and regen-  
950 eration in *osa<sup>308</sup>/+* and wild-type (*w<sup>1118</sup>*) animals. n = 146 wings (*osa<sup>308</sup>/+*) and  
951 296 wings (*w<sup>1118</sup>*) from three independent experiments. Chi-square test  $p < 0.001$   
952 across all wing sizes.

953 (E) Wings were mounted, imaged, and measured after imaginal disc damage and  
954 regeneration in *Bap170 $\Delta$ 135/+* and wild-type (*w<sup>1118</sup>*) animals. n = 100 wings  
955 (*Bap170 $\Delta$ 135/+*) and 224 wings (*w<sup>1118</sup>*) from 3 independent experiments. Student's  
956 t-test, p<0.001

957 (F) Wings were mounted, imaged, and measured after imaginal disc damage and  
958 regeneration in *osa<sup>308/+</sup>* and wild-type (*w<sup>1118</sup>*) animals. n = 142 wings (*osa<sup>308/+</sup>*)  
959 and 284 wings (*w<sup>1118</sup>*) from three independent experiments.

960 (G) Wild-type (*w<sup>1118</sup>*) adult wing after disc regeneration. Anterior is up.

961 (H) *osa<sup>308/+</sup>* adult wing after disc regeneration. Arrows show five anterior-specific  
962 markers in the posterior compartment: anterior crossveins (red), alula-like costa  
963 bristles (orange), margin vein (green), socketed bristles (blue), and change of  
964 wing shape with wider distal portion of the wing, similar to the anterior compart-  
965 ment (purple).

966 (I) Quantification of the number of Posterior-to-Anterior transformation markers  
967 described in (H) in each wing after damage and regeneration of the disc, using  
968 wings that were 75% normal size or larger, comparing *osa<sup>308/+</sup>* wings to wild-type  
969 (*w<sup>1118</sup>*) wings, n = 51 wings (*osa<sup>308/+</sup>*) and 45 wings (*w<sup>1118</sup>*), from 3 independent  
970 experiments. Chi-square test p < 0.001.

971 Error bars are s.e.m. Scale bars are 500 $\mu$ m for all adult wings images. \* p < 0.05,

972 \*\* p < 0.01, \*\*\*p < 0.001 Student's t-test.

973

974 **Fig 3. SWI/SNF core components are required for both growth and poste-**  
975 **rior fate during wing disc regeneration**

976 (A) Comparison of the size of adult wings after imaginal disc damage and regen-  
977 eration in *brm<sup>2/+</sup>* and wild-type (*w<sup>1118</sup>*) animals. n = 142 wings (*brm<sup>2/+</sup>*) and 224

978 wings ( $w^{1118}$ ) from 3 independent experiments, student's t-test  $p < 0.001$ . (A')  
979 Chi-square test  $p < 0.001$  across all wing sizes.

980 (B) Comparison of the size of adult wings after imaginal disc damage and regen-  
981 eration in animals expressing *Bap111* RNAi and control animals.  $n = 264$  wings  
982 (*Bap111* RNAi) and 291 wings (control) from 3 independent experiments. The  
983 control for RNAi lines is VDRC 15293 in all experiments, student's t-test  $p < 0.01$ .  
984 (B') Chi-square test  $p < 0.001$  across all wing sizes.

985 (C-G) Adult wing after disc regeneration of wild-type ( $w^{1118}$ ) (C), *Bap55<sup>LL05955/+</sup>*  
986 (D), *mor<sup>1/+</sup>* (E), RNAi control (F) or *Bap60* RNAi (G). Anterior is up for all adult  
987 wing images. Arrows point to anterior features identified in the posterior compart-  
988 ment. Arrows show five anterior-specific markers in the posterior compartment:  
989 anterior crossveins (red), alula-like costa bristles (orange), margin vein (green),  
990 socketed bristles (blue), and change of wing shape with wider distal portion of the  
991 wing, similar to the anterior compartment (purple).

992 (H) *moira* expression determined by qPCR of *mor<sup>1/+</sup>*, *mor<sup>11/+</sup>* and wild-type  
993 ( $w^{1118}$ ) undamaged wing discs at R24. The graph shows fold change relative to  
994 wild-type ( $w^{1118}$ ) discs.

995 (I) Comparison of the size of adult wings after imaginal disc damage and regen-  
996 eration in *mor<sup>11/+</sup>* and wild-type ( $w^{1118}$ ) animals.  $n = 114$  wings (*mor<sup>11/+</sup>*) and 328  
997 wings ( $w^{1118}$ ) from 3 independent experiments, student's t-test  $p < 0.001$ . (I') Chi-  
998 square test  $p < 0.001$  across all wing sizes.

999 (J) Comparison of the size of adult wings after imaginal disc damage and regen-  
1000 eration in *mor<sup>2/+</sup>* and wild-type ( $w^{1118}$ ) animals.  $n = 134$  wings (*mor<sup>2/+</sup>*) and 414  
1001 wings ( $w^{1118}$ ) from 3 independent experiments, student's t-test  $p < 0.05$ . (J') Chi-  
1002 square test  $p < 0.001$  across all wing sizes.

1003 (K) Comparison of the size of adult wings after imaginal disc damage and regen-  
1004 eration in animals expressing *brm* RNAi and control animals. n = 234 wings (*brm*  
1005 RNAi) and 281 wings (control) from 3 independent experiments, student's t-test p  
1006 < 0.01. (K') Chi-square test p < 0.001 across all wing sizes.

1007 (L) Adult wing after disc regeneration while expressing *brm* RNAi.

1008 (M) Comparison of the size of adult wings after imaginal disc damage and regen-  
1009 eration in *UAS-osa/+; brm<sup>RNAi/+</sup>* and wild-type (*w<sup>1118</sup>*) animals. n = 117 wings  
1010 (*UAS-osa/+; brm<sup>RNAi/+</sup>*) and 348 wings (*w<sup>1118</sup>*) from 3 independent experiments,  
1011 student's t-test not significant. (M') Chi-square test across all wing sizes p =  
1012 0.058, not significant at  $\alpha = 0.05$  level.

1013 (N) Adult wing after imaginal disc regeneration in *UAS-osa/+; brm<sup>RNAi/+</sup>* animal.  
1014 Error bars are s.e.m. Scale bars are 500 $\mu$ m for all adult wing images. \* p < 0.05,  
1015 \*\* p < 0.01, \*\*\*p < 0.001 Student's t-test.

1016

1017 **Fig 4. Decreased *Bap170* expression limits regenerative growth and pupari-**  
1018 **ation delay**

1019 (A) Wild-type (*w<sup>1118</sup>*) regenerating wing disc at R36 with wing pouch marked by  
1020 anti-Nubbin (green) immunostaining.

1021 (B) *Bap170 <sup>$\Delta$ 135/+</sup>* regenerating wing disc at R36 with wing pouch marked by anti-  
1022 Nubbin (green) immunostaining.

1023 (C) Comparison of regenerating wing pouch size at 0, 12, 24, and 36 hours after  
1024 imaginal disc damage in *Bap170 <sup>$\Delta$ 135/+</sup>* and wild-type (*w<sup>1118</sup>*) animals.

1025 (D-E) Regenerating wild-type (*w<sup>1118</sup>*) (D) and *Bap170 <sup>$\Delta$ 135/+</sup>* (E) wing discs at R24  
1026 with Nubbin (green) and PH3 (magenta) immunostaining. Dashed white outline  
1027 shows the regenerating wing primordium labeled with Nubbin.

1028 (F) Average number of mitotic cells (marked with PH3 immunostaining) in the  
1029 wing primordium (marked by anti-Nubbin) at R24 in *Bap170<sup>Δ135/+</sup>* and wild-type  
1030 (*w<sup>1118</sup>*) animals. n = 8 wing discs (*Bap170<sup>Δ135/+</sup>*) and 10 wing discs (*w<sup>1118</sup>*).

1031 (G-H) Wild-type (*w<sup>1118</sup>*) (G) and *Bap170<sup>Δ135/+</sup>* (H) regenerating wing discs at R24  
1032 with Myc immunostaining.

1033 (I) Quantification of anti-Myc immunostaining fluorescence intensity in the wing  
1034 pouch in *Bap170<sup>Δ135/+</sup>* and wild-type (*w<sup>1118</sup>*) regenerating wing discs at R24. n =  
1035 9 wing discs (*Bap170<sup>Δ135/+</sup>*) and 9 wing discs (*w<sup>1118</sup>*).

1036 (L) Median time to pupariation for animals during normal development at 18°C. n  
1037 = 103 pupae (*Bap170<sup>Δ135/+</sup>*) and 227 pupae (*w<sup>1118</sup>*) from 3 independent experi-  
1038 ments. Student's *t*-test not significant.

1039 (M) Median time to pupariation for animals after tissue damage (30°C) and re-  
1040 generation (18°C). n = 117 pupae (*Bap170<sup>Δ135/+</sup>*) and 231 pupae (*w<sup>1118</sup>*) from 3  
1041 independent experiments. Because the temperature shift to 30°C in the ablation  
1042 protocol increases the developmental rate, the pupariation timing of regenerating  
1043 animals (M) cannot be compared to the undamaged control animals (L). stu-  
1044 dent's *t*-test  $p < 0.001$ .

1045 (N) *ilp8* expression examined by qPCR of *Bap170<sup>Δ135/+</sup>* and wild-type (*w<sup>1118</sup>*) re-  
1046 generating wing discs at R24. The graph shows fold change relative to wild-type  
1047 (*w<sup>1118</sup>*) undamaged discs.

1048 (O-Q) Expression of *TRE-Red*, a JNK signaling reporter, in wild-type (*w<sup>1118</sup>*) un-  
1049 damaged (O), as well as wild-type (*w<sup>1118</sup>*) (P) and *Bap170<sup>Δ135/+</sup>* (Q) regenerating  
1050 wing discs at R24. Yellow outline shows the wing disc in (O). White dashed lines  
1051 show the wing pouch in (P) and (Q) as marked by anti-Nub.

1052 (R) Quantification of *TRE-Red* fluorescence intensity in *Bap170 $\Delta$ 135/+* and wild-  
1053 type (*w<sup>1118</sup>*) regenerating wing pouches at R24. n = 12 wing discs (*Bap170 $\Delta$ 135/+*)  
1054 and 14 wing discs (*w<sup>1118</sup>*).

1055 (S) *mmp1* expression examined by qPCR of wild-type (*w<sup>1118</sup>*) and *Bap170 $\Delta$ 135/+*  
1056 regenerating wing discs at R24, and wild-type (*w<sup>1118</sup>*) undamaged discs. The  
1057 graph shows fold change relative to wild-type (*w<sup>1118</sup>*) regenerating discs at R24.  
1058 Scale bars are 100 $\mu$ m for all wing discs images. \* p < 0.05, \*\* p < 0.01, \*\*\* p <  
1059 0.001, Student's *t*-test.

1060

1061

1062 **Fig 5. Reduction of *Osa* causes Posterior-to-Anterior transformations dur-**  
1063 **ing wing disc regeneration**

1064 (A) Wild-type (*w<sup>1118</sup>*) undamaged wing disc with En (green) (A') and Ci (magenta)  
1065 (A'') immunostaining. DNA (blue) (A''') was detected with Topro3 here and in  
1066 subsequent panels. Anterior is left for all wing disc images.

1067 (B) Wild-type (*w<sup>1118</sup>*) regenerating wing disc at R72 with En (green) (B') and Ci  
1068 (magenta) (B'') immunostaining and DNA (blue) (B''').

1069 (C) *osa<sup>308/+</sup>* regenerating wing disc at R72 with En (green) (C') and Ci (magenta)  
1070 (C'') immunostaining, and DNA (blue) (C'''). Arrowhead points to the low En ex-  
1071 pression region in which Ci is expressed in the posterior compartment.

1072 (D) Wild-type (*w<sup>1118</sup>*) undamaged wing disc with Ac immunostaining.

1073 (E) Wild-type (*w<sup>1118</sup>*) regenerating wing disc at R72 with Ac immunostaining.

1074 (F) *osa<sup>308/+</sup>* regenerating wing disc at R72 with Ac immunostaining. Arrowheads  
1075 show Ac expression in the posterior compartment.

1076 Scale bars are 100 $\mu$ m for all wing discs images.

1077



1078 **Fig 6. The BAP complex is required to maintain posterior cell fate during**  
1079 **wing disc regeneration**  
1080 (A) Wild-type ( $w^{1118}$ ) undamaged wing disc with Ptc (green) (A') and Ci (magenta)  
1081 (A'') immunostaining.  
1082 (B) Wild-type ( $w^{1118}$ ) regenerating wing disc at R72 with Ptc (green) (B') and Ci  
1083 (magenta) (B'') immunostaining.  
1084 (C)  $osa^{308/+}$  regenerating wing disc at R72 with Ptc (green) (C') and Ci (magenta)  
1085 (C'') immunostaining. Arrowhead shows Ptc and Ci co-expression in the posterior  
1086 compartment.  
1087 (D) RNAi control regenerating wing disc at R72 with Ptc (green) (D') and Ci (ma-  
1088 genta) (D'') immunostaining.  
1089 (E) Regenerating wing disc of animals expressing *brm* RNAi at R72 with Ptc  
1090 (green) (E') and Ci (magenta) (E'') immunostaining. Arrowheads show Ptc and Ci  
1091 co-expression in the posterior compartment.  
1092 Scale bars are 100 $\mu$ m for all wing disc images.

1093

1094 **Fig 7. The BAP complex functions in parallel to Tara to prevent P-to-A**  
1095 **transformations.**

1096 (A-B) Expression of *TRE-Red*, a JNK signaling reporter, in wild-type ( $w^{1118}$ ) (A)  
1097 and  $osa^{308/+}$  (B) regenerating wing discs at R24. Dashed white outline shows the  
1098 regenerating wing primordium as marked by anti-Nub and excluding the debris  
1099 field.  
1100 (C) Quantification of *TRE-Red* expression fluorescence intensity in  $osa^{308/+}$  and  
1101 wild-type ( $w^{1118}$ ) regenerating wing pouches at R24. n = 26 wing discs ( $osa^{308/+}$ )  
1102 and 31 wing discs ( $w^{1118}$ ). Error bars are s.e.m.

1103 (D-F) Tara expression detected with anti-  $\beta$ -gal immunostaining in *tara-lacZ/+* un-  
1104 damaged (D), *tara-lacZ/+* R48 (E) and *Bap55<sup>LL05955/+</sup>; tara-lacZ/+* R48 (F) regen-  
1105 erating wing discs.

1106 (G) Quantification of  $\beta$ -gal expression via fluorescence intensity to determine lev-  
1107 els of *tara-lacZ* expression in *Bap55<sup>LL05955/+</sup>* and wild-type (*w<sup>1118</sup>*) regenerating  
1108 wing pouches at R48. n = 8 wing discs (*Bap55<sup>LL05955/+</sup>*) and 9 wing discs (*w<sup>1118</sup>*).  
1109 Error bars are s.e.m.

1110 (H-J) Adult wings after disc regeneration in wild-type (*w<sup>1118</sup>*) (H), *osa<sup>308/+</sup>* (I) and  
1111 *UAS-tara/+; osa<sup>308/+</sup>* (J) animals. Arrows show five anterior-specific markers in  
1112 the posterior compartment: anterior crossveins (red), alula-like costa bristles (or-  
1113 ange), margin vein (green), socketed bristles (blue), and change of wing shape  
1114 with wider distal portion of the wing, similar to the anterior compartment (purple).  
1115 Anterior is up for all adult wing images.

1116 (K) Quantification of the number of Posterior-to-Anterior transformation markers  
1117 described above in each wing after damage and regeneration of the disc, com-  
1118 paring *UAS-tara/+; osa<sup>308/+</sup>* wings to *osa<sup>308/+</sup>* and wild-type (*w<sup>1118</sup>*) wings, n = 21  
1119 wings (*UAS-tara/+; osa<sup>308/+</sup>*), n = 16 wings (*osa<sup>308/+</sup>*) and n = 34 wings (*w<sup>1118</sup>*),  
1120 from 3 independent experiments. \*\*\* p < 0.001, Chi-square test. Chi-square test  
1121 measuring *UAS-tara/+; osa<sup>308/+</sup>* against *w<sup>1118</sup>*, p = 0.86, is not significant.

1122 Scale bars are 100 $\mu$ m for all wing discs images. Scale bars are 500 $\mu$ m for all  
1123 adult wings images. \* p < 0.05, \*\* p < 0.01, Student's *t*-test for (C) and (G).

1124

1125 **Fig 8. Cell fate changes induce ectopic AP boundaries in the posterior com-**  
1126 **partment during wing disc regeneration**

1127 (A) Wild-type (*w<sup>1118</sup>*) undamaged wing disc with Ptc (green) (A') and pSMAD (ma-  
1128 genta) (A'') immunostaining.

- 1129 (B) Wild-type ( $w^{1118}$ ) regenerating wing disc at R48 with Ptc (green) (B') and  
1130 pSMAD (magenta) (B'') immunostaining.
- 1131 (C)  $osa^{308/+}$  regenerating wing disc at R48 with Ptc (green) (C') and Ci (magenta)  
1132 (C'') immunostaining.
- 1133 (D) Proposed working model for the functions of the PBAP and BAP complexes  
1134 in regeneration.
- 1135

Fig 1

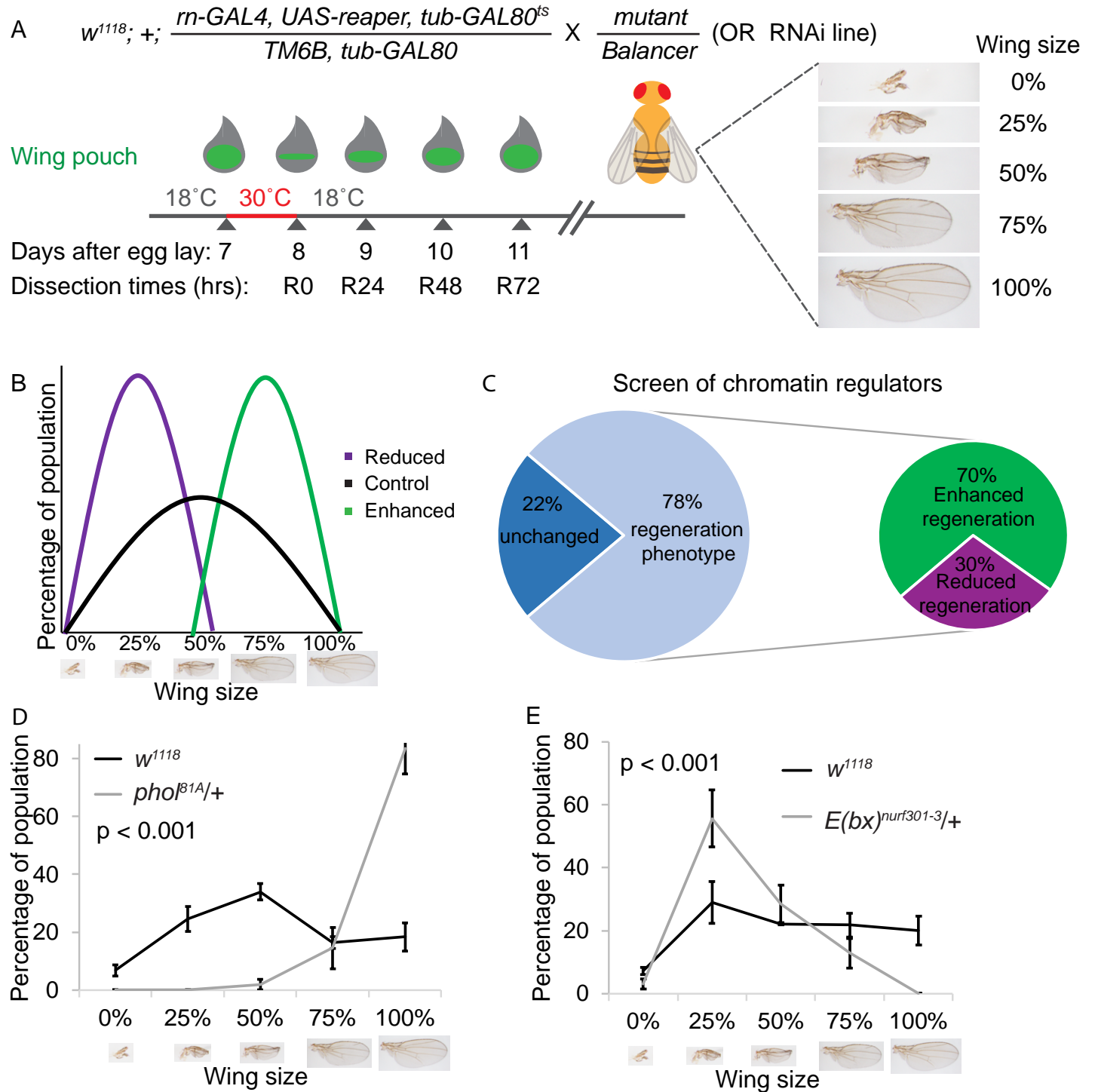
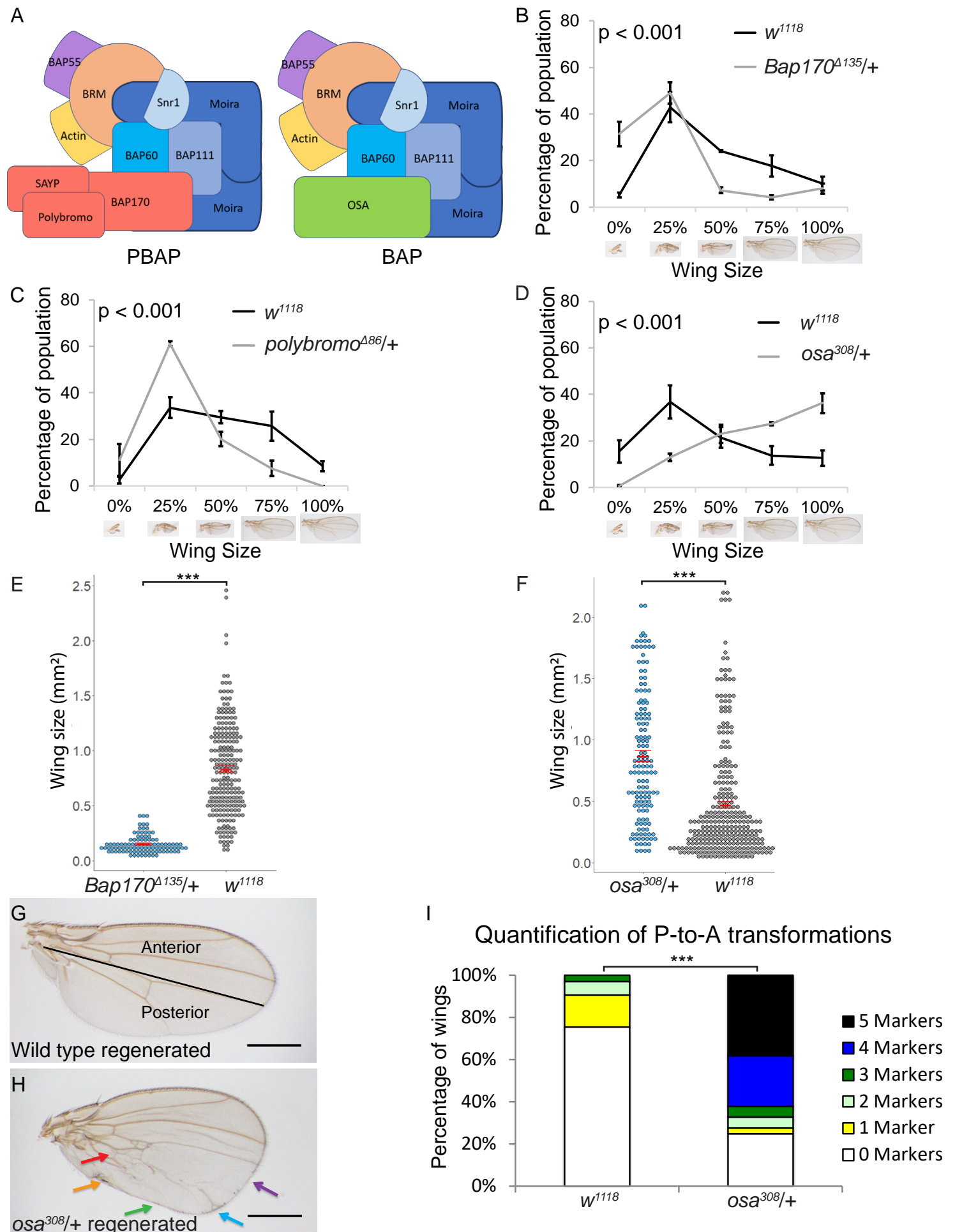


Fig 2



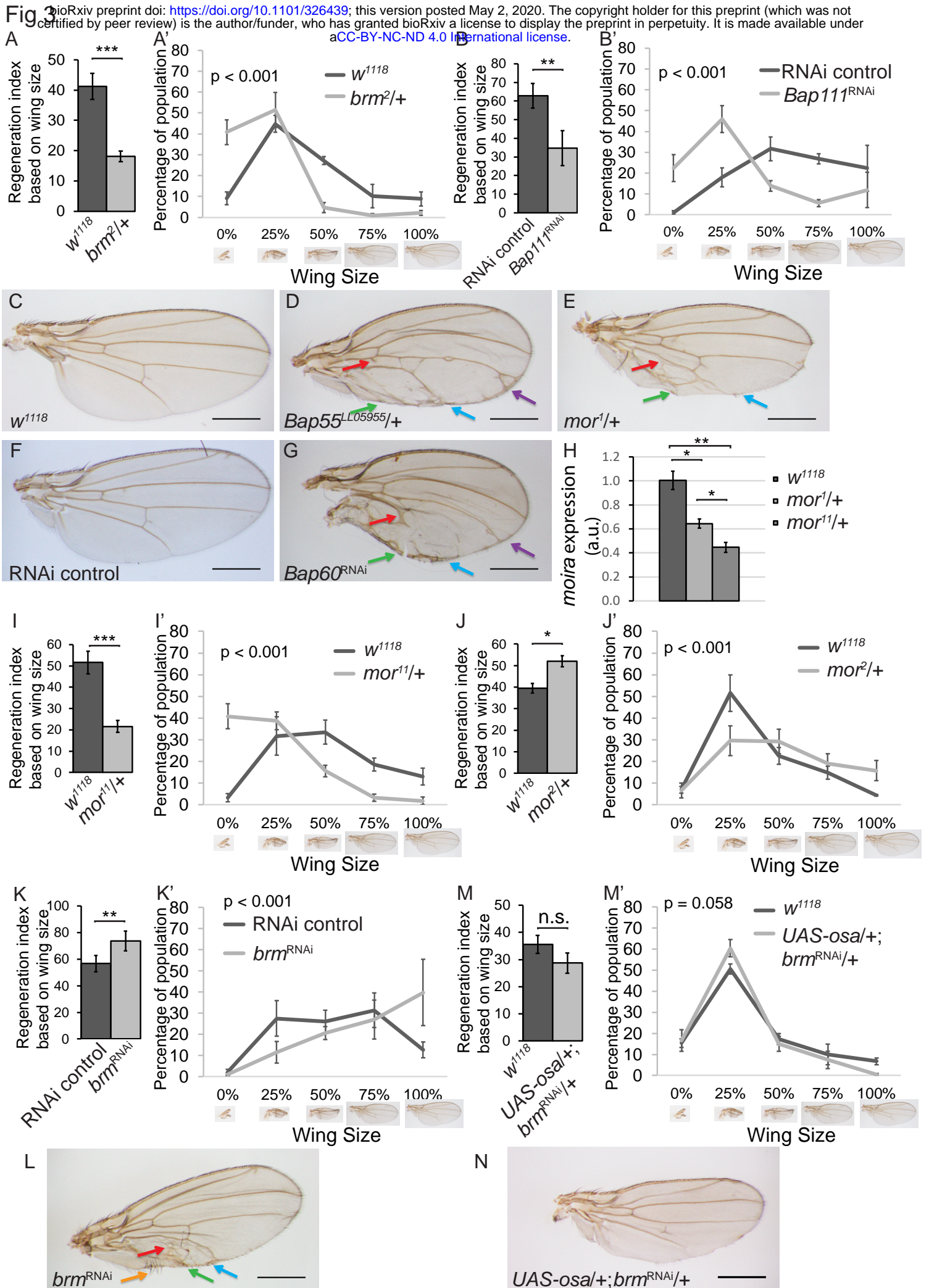


Fig 4

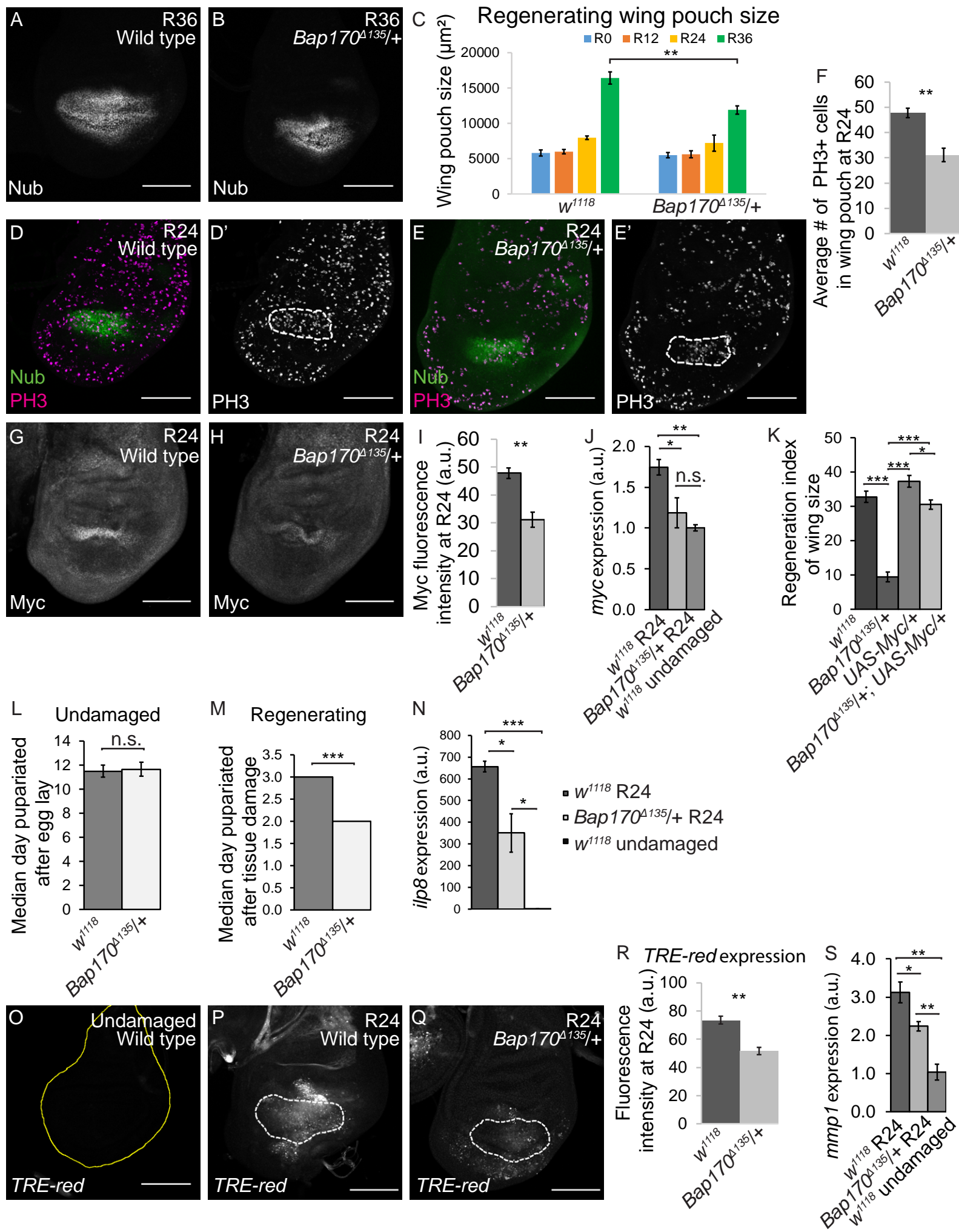


Fig 5

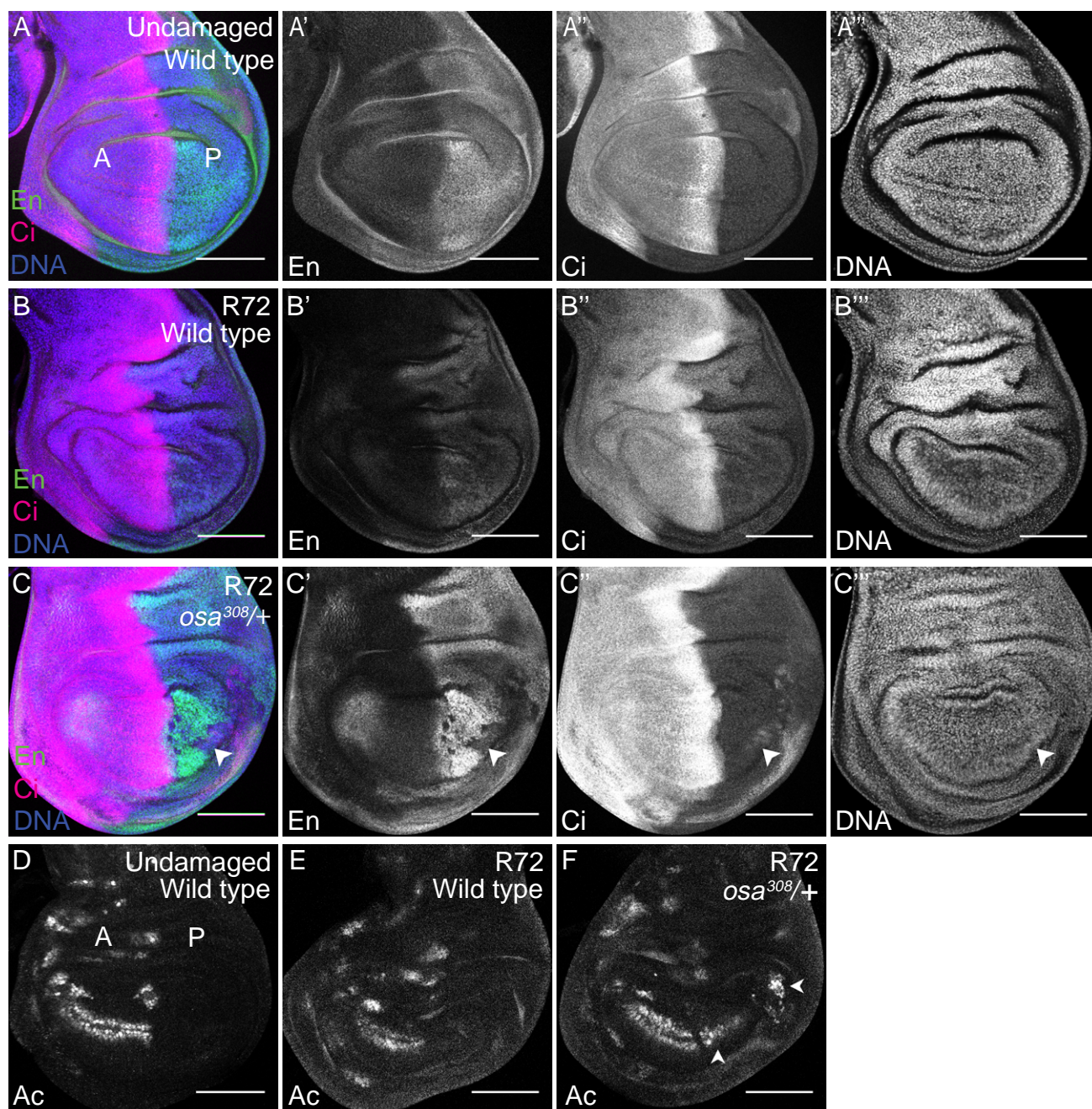




Fig 6

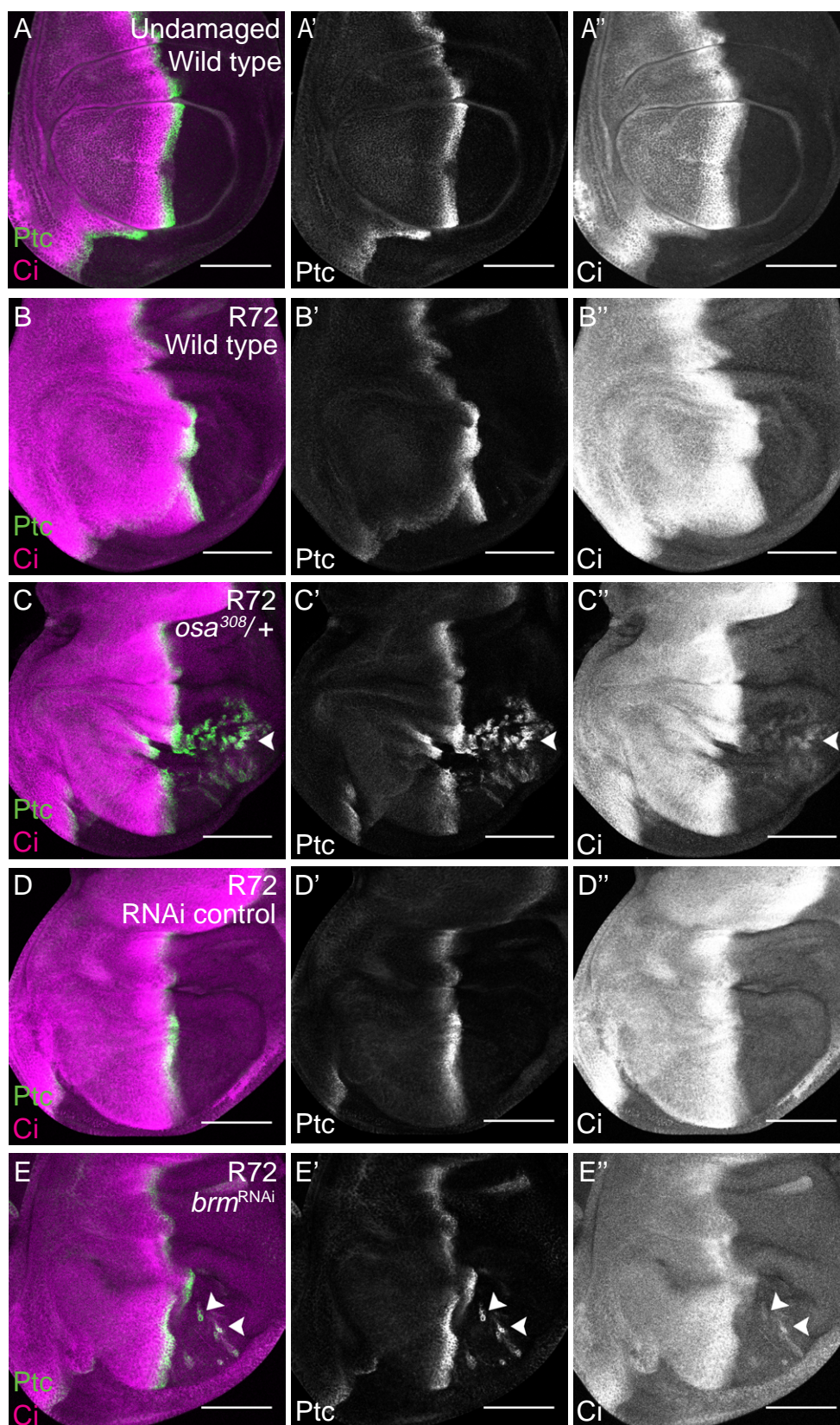


Fig 7

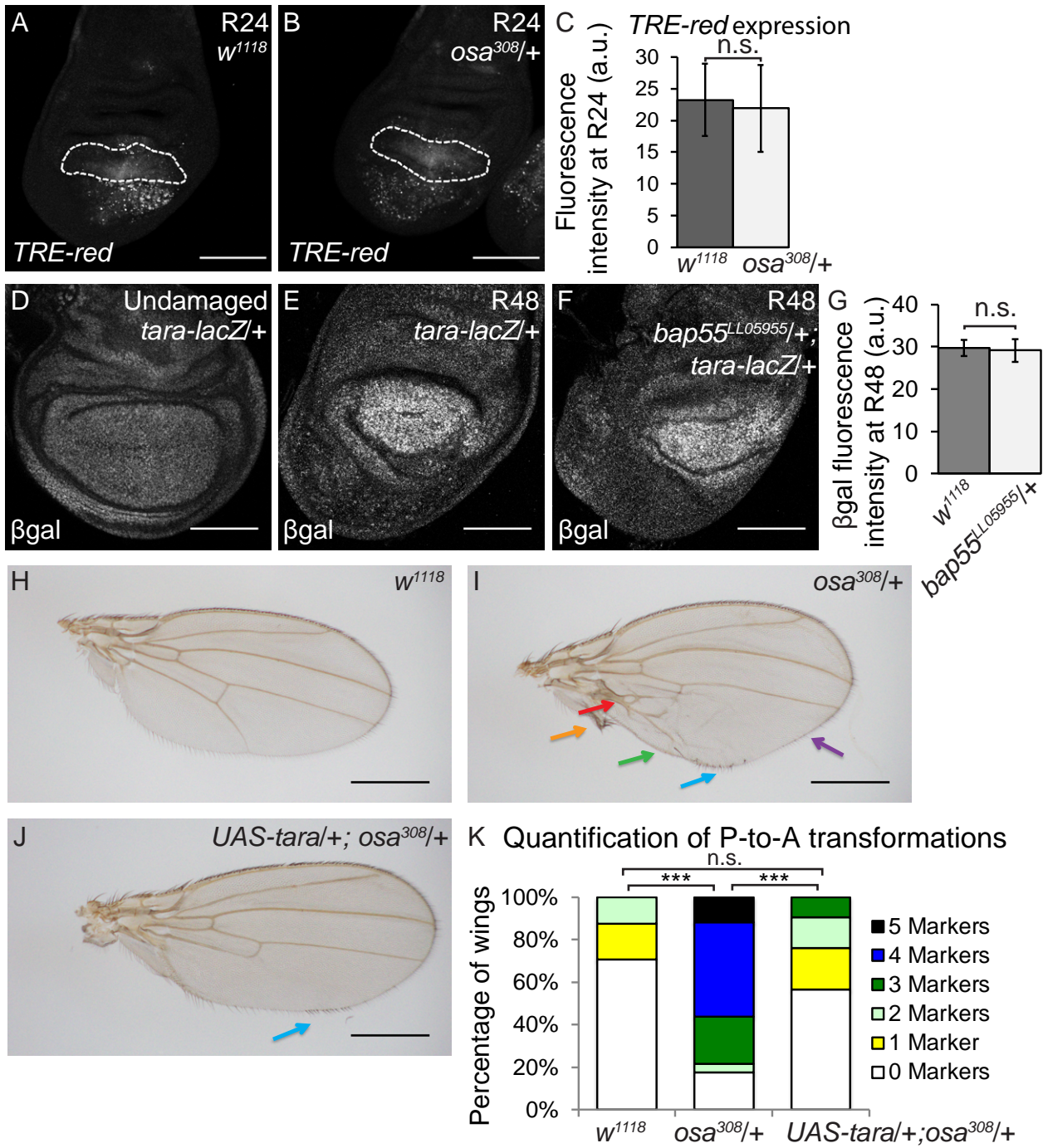


Fig 8

