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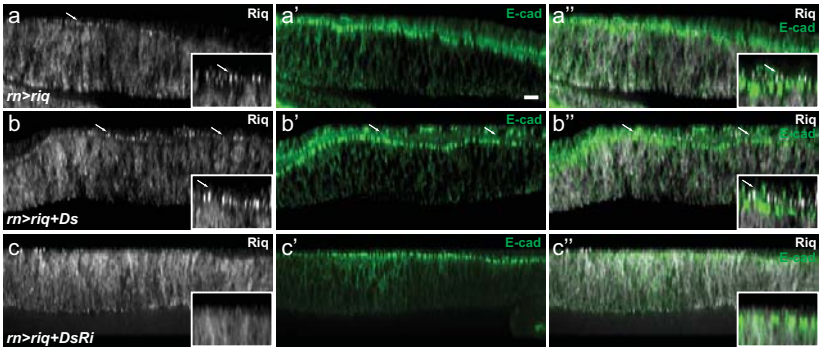


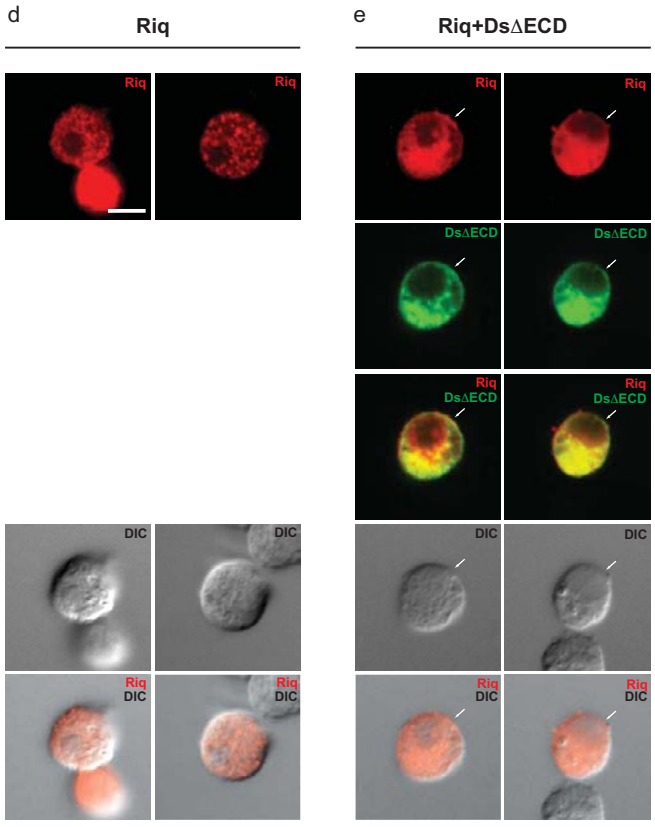
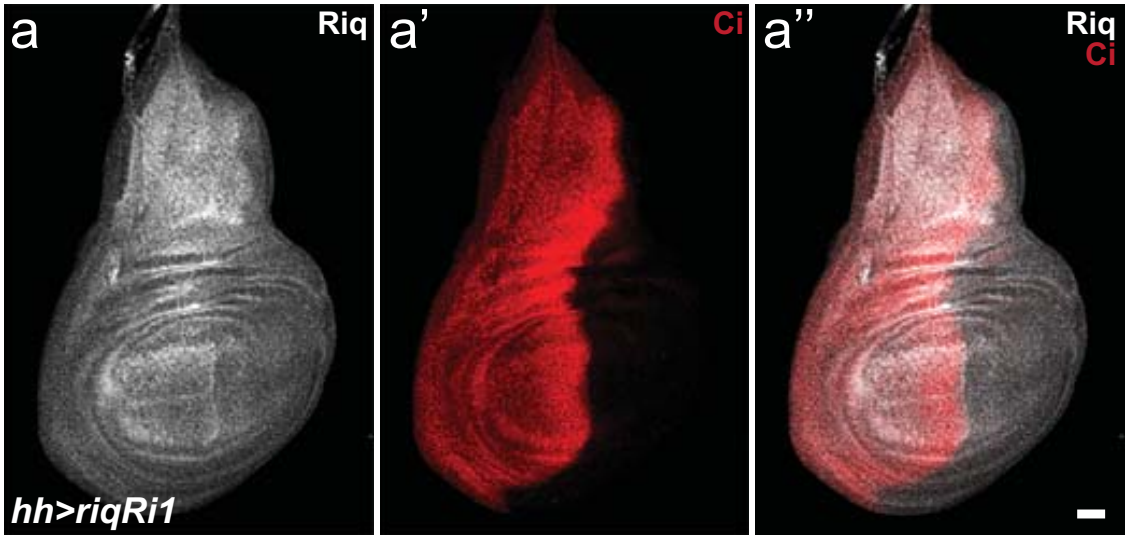


Figure S1 Dachsous promotes localization of Riquiqui to apical junctions and cell membranes. (a-c") X-Z cross-sections of *D. melanogaster* third instar larval wing imaginal discs expressing the following elements: (a, a', a") *rn-Gal4, UAS-riq;* (b, b', b") *rn-Gal4, UAS-riq* and *UAS-ds;* (c, c', c") *rn-Gal4, UAS-riq* and *UAS-dsRi*. Riq is in white, E-cadherin marks the adherens junction in green. Arrows indicate Riq protein that is present at apical junctions in Ds-expressing tissues. Magnified images are inset in (a) (a') (b) and (b'). Merged images are on the right. Riq was junctionally localised immediately apical to E cadherin. Riq was largely absent from apical junctions of tissues where Ds was depleted by RNAi. (d and e) *D. melanogaster* S2 cells expressing either Riq (d) or Riq and DsΔECD (e). Riq is in red, DsΔECD is green, Direct interference contrast (DIC) images are grey. Arrows indicate Riq protein that is present at cell membranes in DsΔECD-expressing cells. Scale bar represents 10μm in (a-c") and 5μm in (d) and (e).



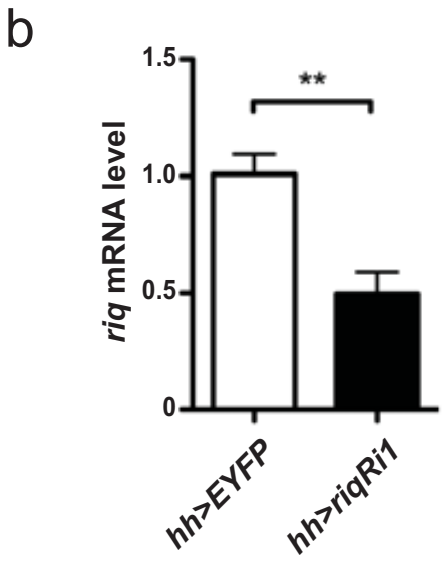


Figure S2 *UAS-riq RNAi* transgenes decrease Riq expression. (a) A wing imaginal disc from a third instar *D. melanogaster* larva expressing *UAS-riqi1* under the control of *hh-Gal4*. Riq expression (grey in a) was assessed using an antibody directed against DCAF7 (human homologue of Riq). Cubitus interruptus (Ci) expression (red in a') marked the anterior compartment of wing imaginal discs. A merged image is shown in (a''). (b) The level of *riq* mRNA relative to the control *actin5C* mRNA, assessed by QRT-PCR, in wing imaginal discs harbouring the following transgenes: *hh-Gal4* and *UAS-EYFP*; or *hh-Gal4 and UAS-riqi1*. Data is presented as mean ± SEM, n=3, ** = p<0.01. Scale bar represents 50μm in (a). Statistics source data for Fig. S2b is available in Supplementary Table S1.

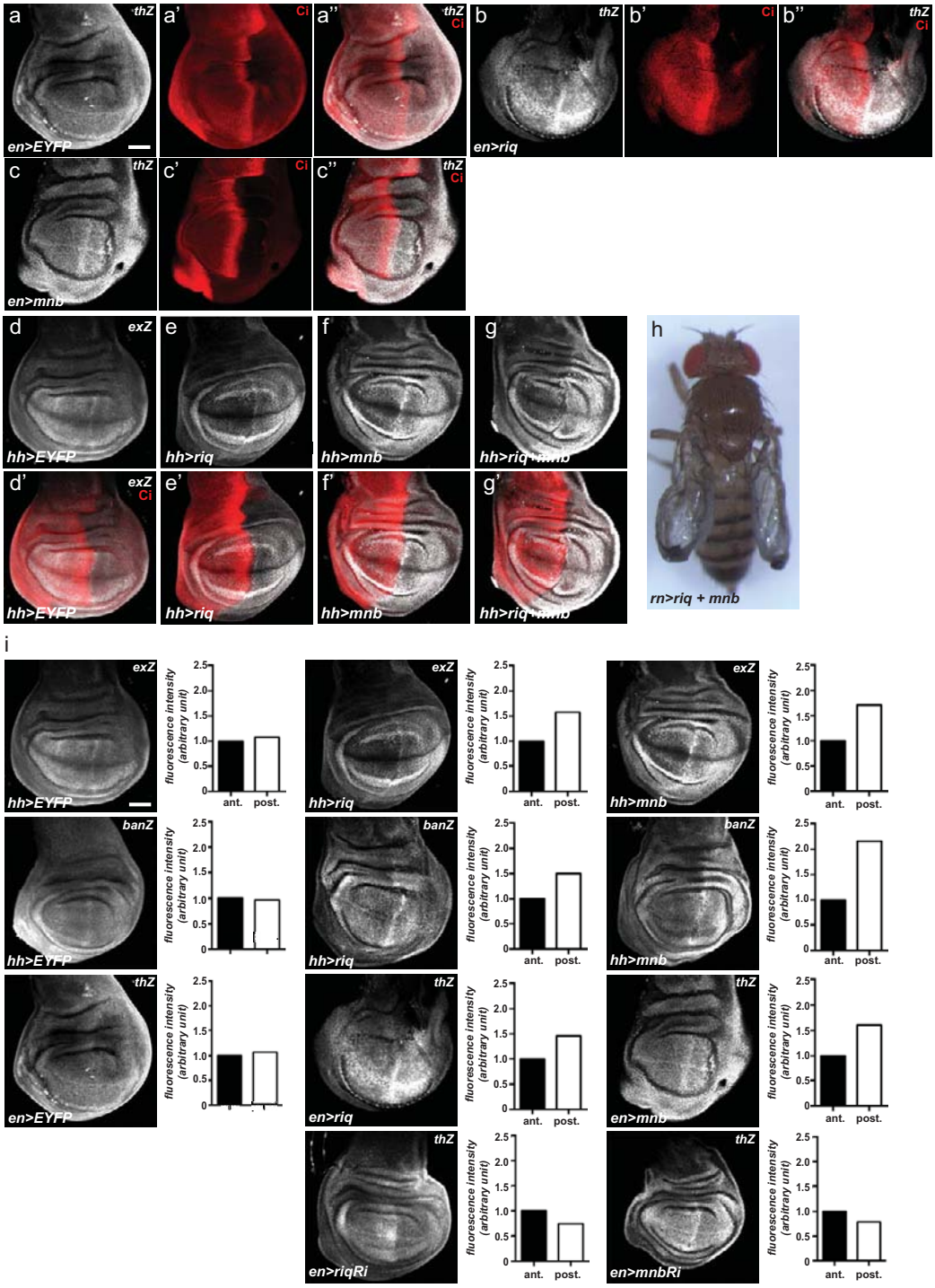


Figure S3 Riquiqui and Minibrain regulate SWH pathway activity. Wing imaginal discs from third instar *D. melanogaster* larvae harbouring the following elements: (a-a'') *en-Gal4, UAS-EYFP* and *th-lacZ;* (b-b'') *en-Gal4*, *UAS-riq* and *th-lacZ*; (c-c'') *en-Gal4, UAS-mnb* and *th-lacZ*; (d, d') *hh-Gal4, UAS-EYFP* and *ex-lacZ;* (e, e') *hh-Gal4, UAS-riq* and *ex-lacZ*; (f, f') *hh-Gal4, UAS-mnb* and *ex-lacZ*; (g, g') *hh-Gal4, UAS-riq, UAS-mnb* and *ex-lacZ*. Yki activity (grey) was reported by *th-lacZ* (a-c) or *ex-lacZ* (d-g). All transgenes were expressed in the posterior compartment of wing imaginal discs; Ci expression (red) marks the anterior compartment. Merged images are shown in (a''-c'' and in d'-g'). (h) An adult female fly expressing both *riq* and *mnb* under control of the *rn-Gal4* driver. Unlike driving expression of *riq* and *mnb* alone, driving expression of both transgenes together caused severe folding and crumpling of wings, which prevented quantification of their size. (e) Quantification of LacZ expression driven by *ex-lacZ, ban-lacZ* or *th-lacZ* in wing imaginal discs displayed in Fig. 3 and Supplementary Fig. S3a-c. Anterior is to the left. For each disc fluorescence intensity was measured by ImageJ in two boxes of defined size in both the anterior (ant.) and posterior (post.) compartments. These values were averaged and plotted for each experiment. Scale bar represents 50μm.

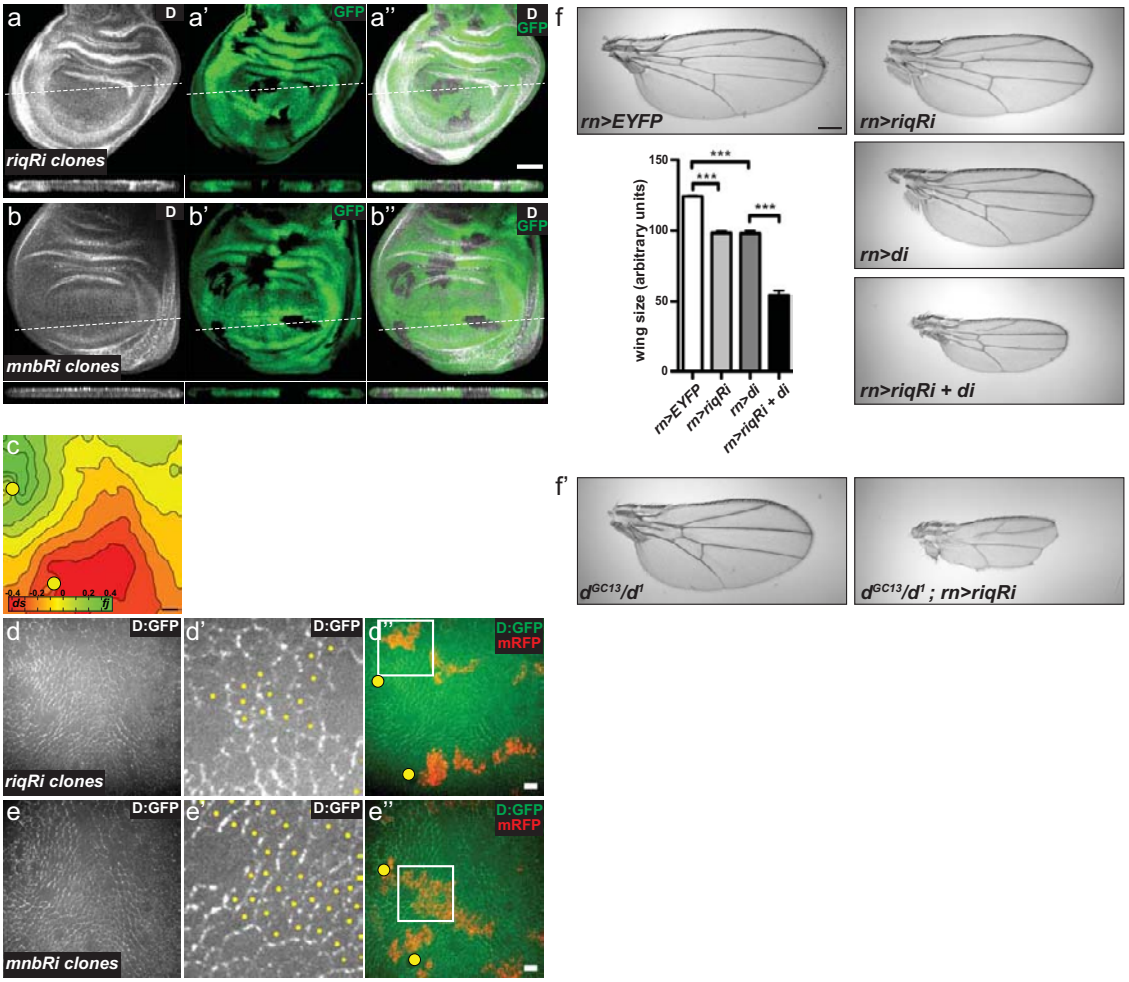


Figure S4 Riquiqui and Minibrain affect wing growth independently of Dachs. Wing imaginal discs from third instar *D. melanogaster* larvae containing clones of *UAS-riqi1* (a) or *UAS-mnbi1* (b) were stained for Dachs (grey in a and b). Clones were positively marked by co-expression of GFP (a' and b'). Merged images are shown in (a'' and b''). X-Z sections through the wing pouch are shown below planar sections of wing discs in a-b''. Dashed lines represent the section used to obtain X-Z images. No changes in Dachs expression or localization were observed. (d-e'') All images were recorded in pupal scutellum tissue (right-sided hemi-scutellum) at 20 hour after pupa formation. Anterior is to the right, the midline is to the top. Yellow circles mark the macrocheatae. Spatial information on the Fj-Ds gradient is shown in c as in[1]. Flip out clones of *UAS-riqi* and *UAS-mnbi* were generated and marked in red (d'' and e'' respectively). White boxes are magnified in d' and e'. Yellow dots mark the mutant cells abutting wt cells (d' and e'). In these Flip-Out clones no clear reduction of D:GFP levels was observed when compared with the surrounding tissue. There is also no clear repolarization of D:GFP near clone boundaries. (f) Wing area from adult female flies expressing *UAS-EYFP* as control, *UAS-riqi*, *UAS-dachs RNAi (di)*, *UAS-riqi* concomitantly with *UAS-di*, under the control of *rn-Gal4* (n=20 for each genotype). Representative images of each genotype are shown. Data is presented as mean ± SEM, *** = p<0.001. These results suggest Dachs and Riq control tissue growth by acting in parallel. Scale bars represents 50μm in (a) and (b), 10μm in (c), (d) and (e), and 200μm in (f).

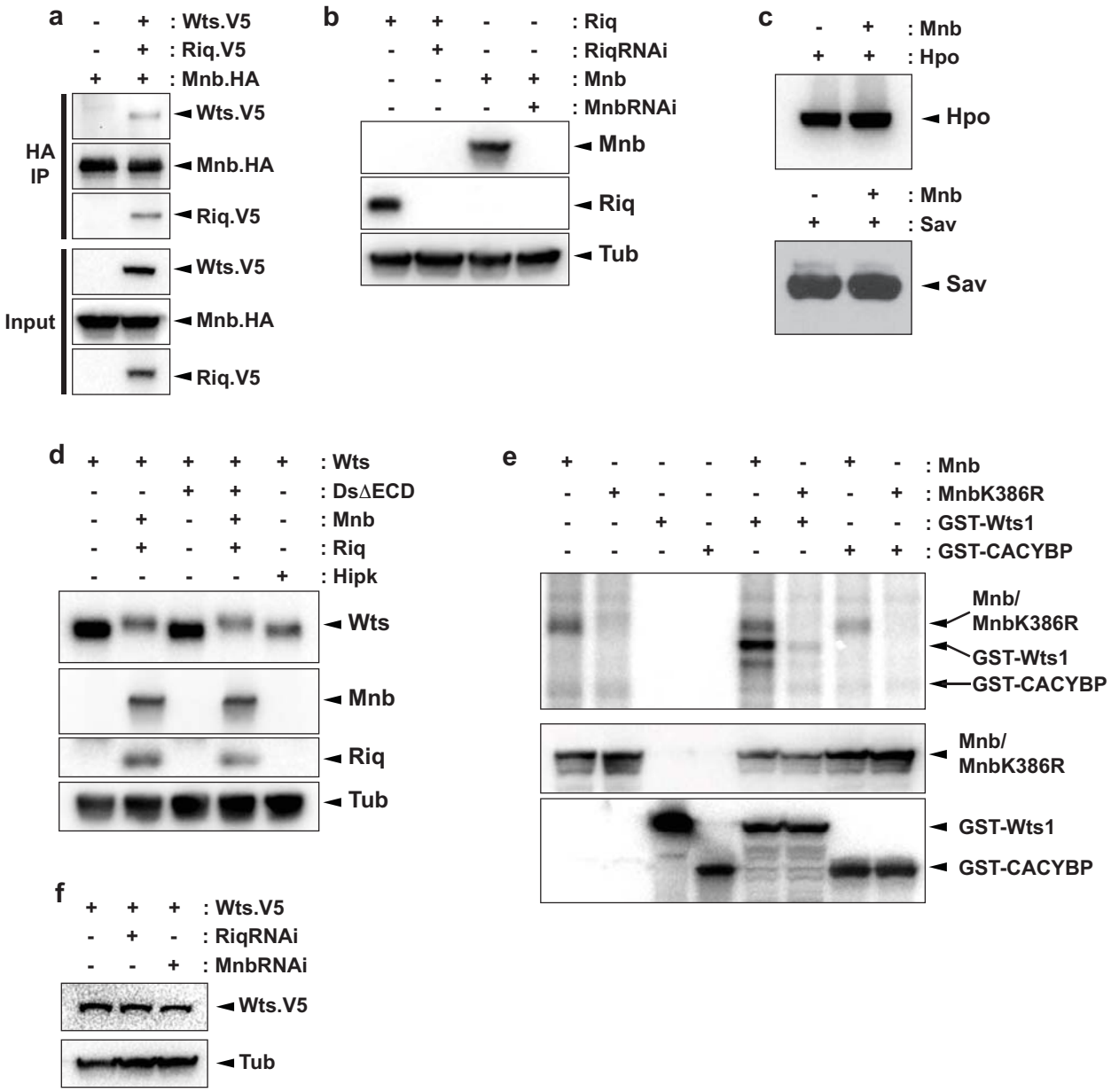


Figure S5 Riquiqui, Minibrain and Warts physically interact with each other and Minibrain phosphorylates Warts. (a) S2 cells were transfected with the indicated plasmids and immunoprecipitations performed using anti-HA antibodies. Subsequently, immunoprecipitates and input lysates were subjected to SDS-PAGE and Western blotted to reveal the indicated proteins. (b-d) Western blot analysis of lysates from S2 cells transfected with the indicated plasmids and Western blotted to reveal the indicated proteins. In (b) Riq or Mnb were expressed in S2 cells in the presence or absence of dsRNA specific for Riq or Mnb, respectively. In (c) Mnb did not induce changes in Hpo or Sav mobility. In (d) Mnb, but not Hipk influenced Wts mobility. (e) Kinase assays performed using recombinant GST-Wts1 or GST-CACYBP as substrates and either Mnb or Mnb K386R immunoprecipitated from S2 cells. Immunopurified and recombinant proteins were incubated alone or together in kinase buffer containing γ^{32}P-ATP and subjected to SDS-PAGE (upper panel). Western blotting was used to detect input proteins (lower panels). Mnb phosphorylated GST-Wts1 but not the negative control substrate GST-CACYBP. (f) V5-tagged Wts was expressed in the presence or absence of dsRNA specific for Riq or Mnb in S2 cells. Cells were lysed and lysates were immunoblotted using antibodies to V5 and to Tubulin as a loading control. Wts levels were unchanged when Riq or Mnb were depleted from cells by RNAi.

a

Experiment 1 - Wild-type Mnb		Experiment 1 - Kinase-dead Mnb
Phosphorylation site	Wts Peptide	
T59	R.NDAL**T***PDYHHAK.Q	**No Phosphorylation detected**
T173	R.**T***VGNPGGNGGFSPSPSGFSEVAPPAPPPR.N	
S184	R.TVGNPGGNGGF**S***PSPSGFSEVAPPAPPPR.N	
S227	K.RR**S***PALNNRPPAIAPPTQR.G	
S246	R.GN**S***PVITQNGLK.N	

b

Experiment 2 - Wild-type Mnb		Experiment 2 - Kinase-dead Mnb	
Phosphorylation site	Wts Peptide	Phosphorylation site	Wts Peptide
T59	R.NDAL**T***PDYHHAK.Q	S250	R.GNSPVIT*QNGLKNPQQQLTQQLK.S
S146	R.C**S***PALDSGAGSSR.S	S313	R.QS*PTQSQVDSSGR.I
S227	R.**S***PALNNRPPAIAPPTQR.G		
S246	R.GN**S***PVITQNGLK.N		
S250	R.GNSPVIT*QNGLKNPQQQLTQQK.S		
S313	R.QS*PTQSQVDSSGR.I		

c

Wts residues phosphorylated by Wild-type Mnb but not kinase-dead Mnb

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MHPAGEKRGGRPNDKYTAEALESIKQDLTRFEVQNNHRNNQNYTPLRYTATNGRNDALTP 60
*: . : .* *     *  * :      ::  :*: :.. :* :    ::.  :.    .

DYHHAKQPMEPPPSASPAPDVVIPPPPAIVGQPGAGSISVSGVGVG-VVGVANGRVPKMM 119
.  :      *  .    .:     ::   .   . * .   ..      : : . :

TALMPNKLIR------KPSIERDTASSHYLRCSPALDSGAGSSRSDSPH---SHHTHQPS 170
:: :                    ..       :   :. . . : :   . : ::.

SRTVGNPGGNGGFSPSPSGFSEVAPPAPPPRNPTACSAATPPPPVPPTSQAYVKRRSPAL 230
*  .  *.: .: *.  .          . .* . :      * .           .:

NNRPPAIAPPTQRGNSPVITQNGLK------NPQQQLTQQLKSLNLYPGGGSGAVVEPPP 284
.:*  .  .   *. :*                     :   . : *.*. .  : . .*

PYLIQGGAGGAAPPPPPPSYTASMQSRQSPTQSQ                          318
:  *.   .              *
```

Figure S6 Minibrain phosphorylates six amino acids in the Warts protein, including a DYRK1A consensus site. (a and b) Wts1 (amino acids 1-318 of Wts) peptides that possessed amino acids that were phosphorylated when incubated with either wild-type Mnb or kinase-dead Mnb (Mnb-KD) immunoprecipitates. Experiments 1 and 2 were performed independently and analyzed by different mass spectrometry facilities. In experiment 1, five Wts amino acids were phosphorylated by wild-type Mnb with high confidence, whereas Mnb-KD induced no observable phosphorylation. In experiment 2, six Wts amino acids were phosphorylated by wild-type Mnb with high confidence, whilst two amino acids were phosphorylated in the presence of Mnb-KD. Amino acids that were phosphorylated by Mnb, but not Mnb-KD, are highlighted; amino acids that were phosphorylated in both independent experiments are red and amino acids that were phosphorylated in only one experiment are blue. (c) A summary of mass spectrometry data outlined in (a) and (b). The amino acid sequence of Wts1 is shown and the degree of conservation with human LATS1, as assessed by CLUSTAL, is indicated below: * indicates perfect conservation, : indicates strongly similar; . indicates weakly similar. As in (a) and (b), amino acids that were phosphorylated in both independent experiments by Mnb, but not Mnb-KD, are highlighted in red and amino acids that were phosphorylated by Mnb in only one experiment are in blue. The Wts sequence that was phosphorylated by Mnb and closely resembles a DYRK1A consensus site $(RPXS/TP)^2$ is in bold font. Of note, this site was phosphorylated in each experiment and is conserved between Wts and LATS1.

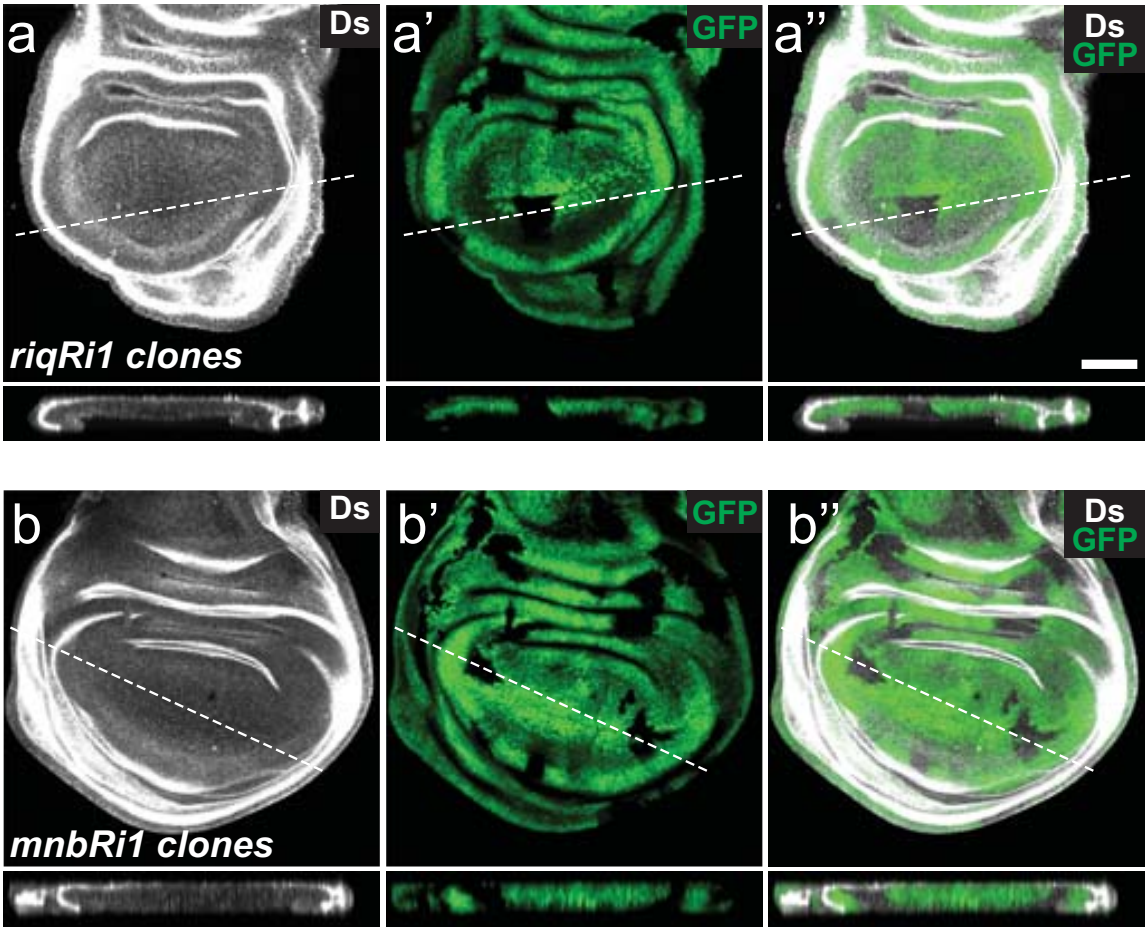


Figure S7 Reduction of Riq or Mnb expression does not affect Dachsous expression. Wing imaginal discs from third instar *D. melanogaster* larvae containing clones of *UAS-riqi1* (a) or *UAS-mnbi1* (b) were stained for Ds (grey in a and b). Clones were positively marked by co-expression of GFP (a' and b'). Merged images are shown in (a" and b"). X-Z sections through the wing pouch are shown below planar sections of wing discs in a-b". Dashed lines represent the section used to obtain X-Z images. Scale bar represents 50μm.

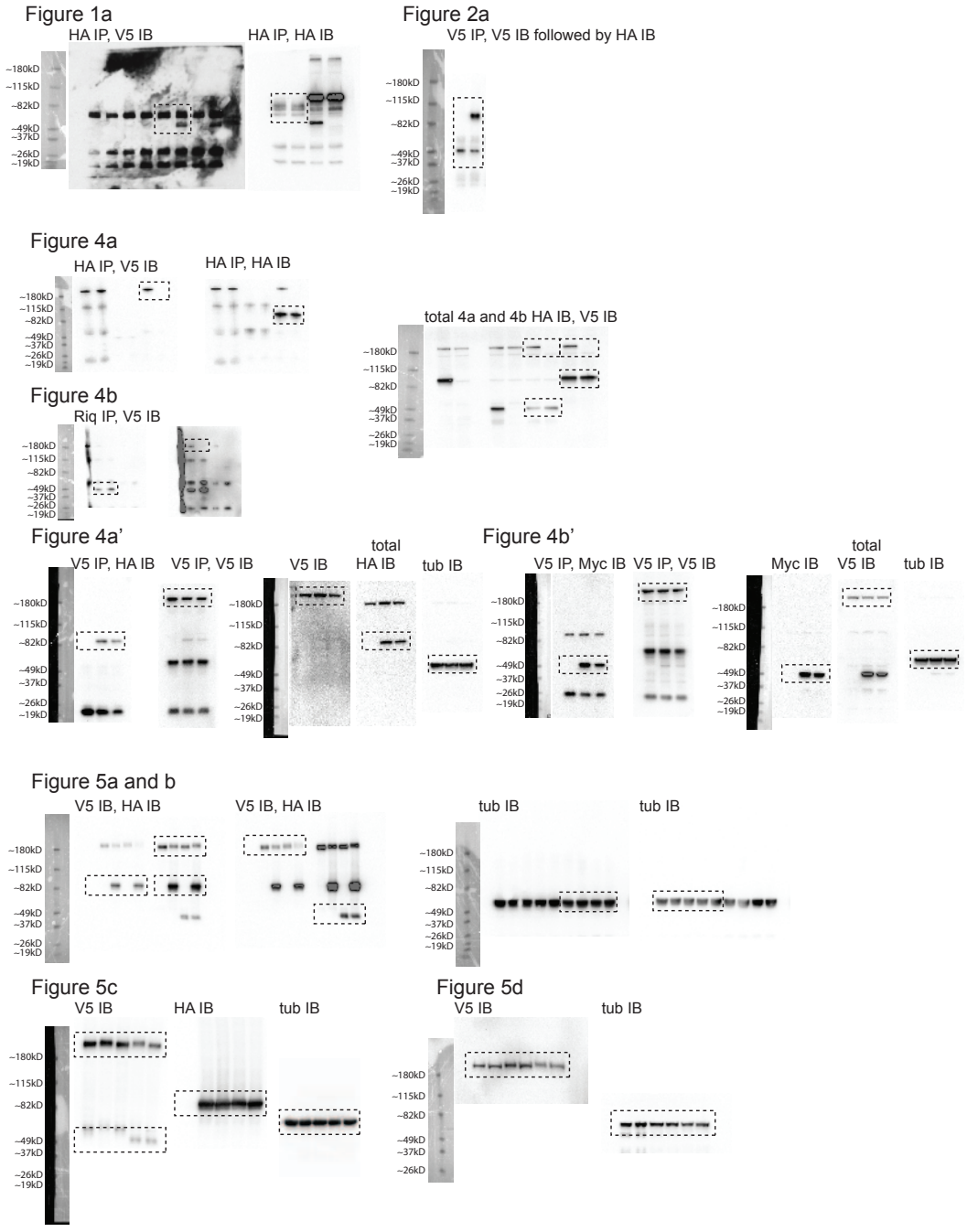


Figure S8 Full scan

Figure 5f

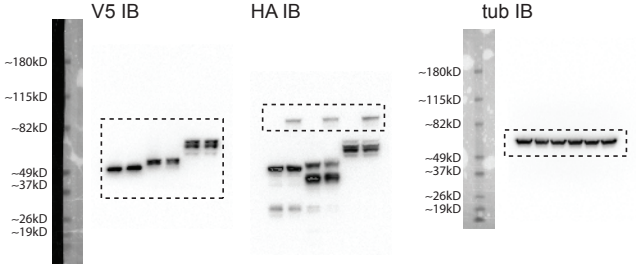


Figure 5g

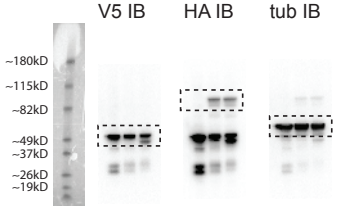


Figure 5h

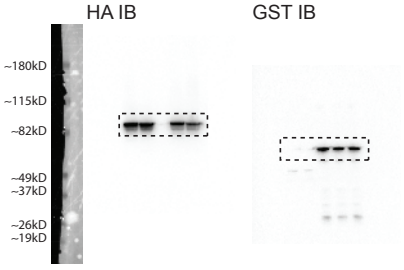
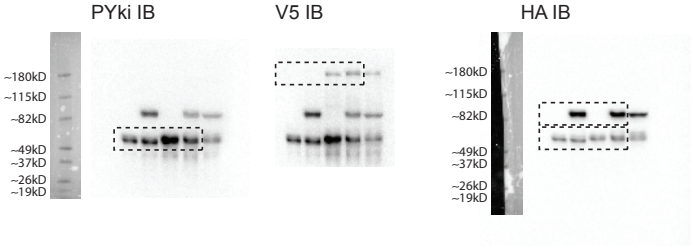


Figure 5i



Supplementary References

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2. Himpel, S. *et al*. Specificity determinants of substrate recognition by the protein kinase DYRK1A. *J Biol Chem* **275**, 2431-2438 (2000).