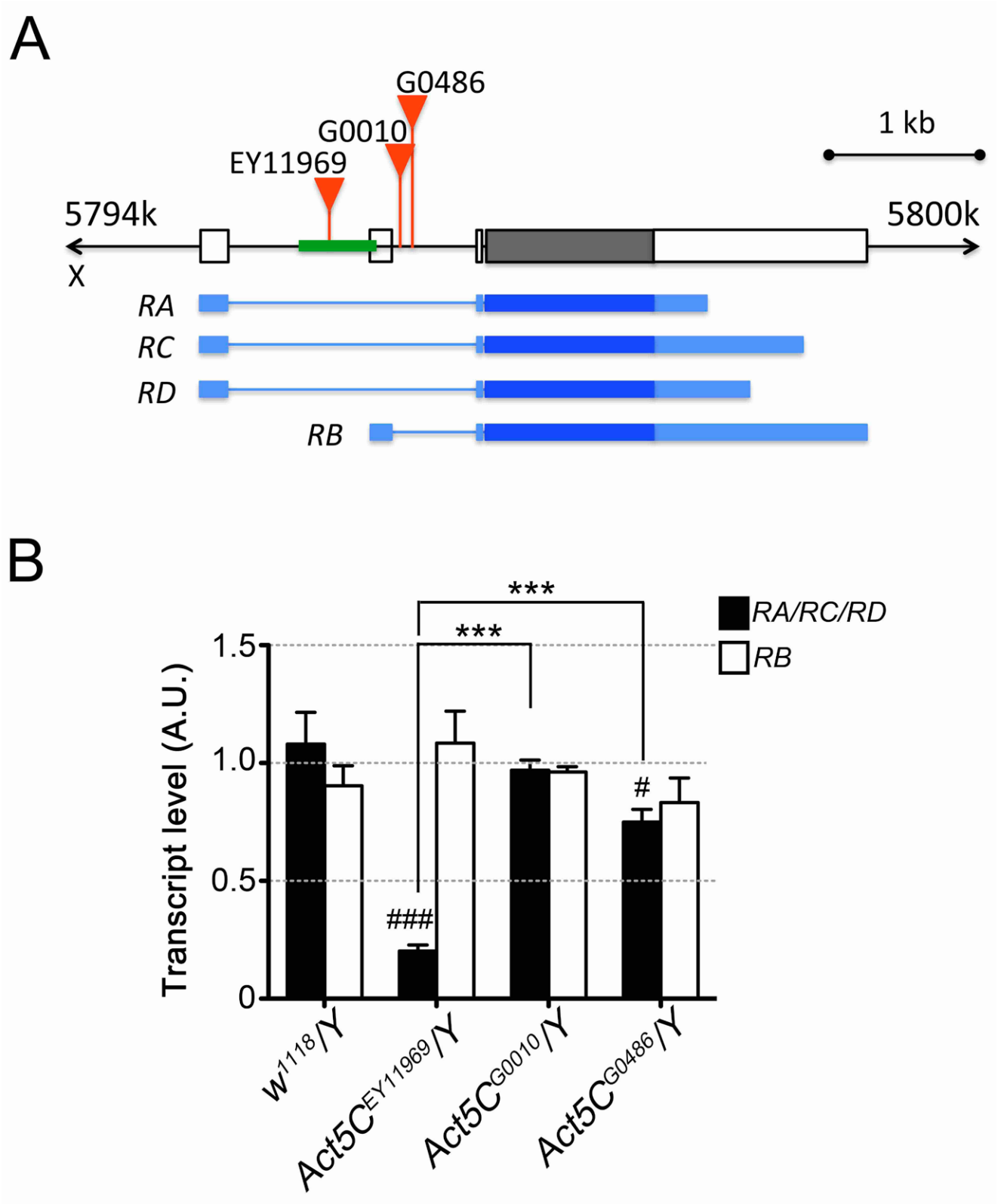

Fig. S1. *Act5C* expression is reduced in *EY11969* flies. (A) Schematic diagram of the *Act5C* genomic region. The top panel shows the insertion positions of P-element lines *EY11969*, *G0010* and *G0486*. Boxes represent exons, and the filled box in exon 3 represents the coding region. The proximal promoter is highlighted in green. The bottom panel shows the isoforms generated by alternative splicing. Dark blue boxes represent the ORFs. (B) qRT-PCR analysis of *Act5C* isoform expression in adult head extracts of *EY11969*, *G0010* and *G0486* male flies. Average (mean ± SEM) is shown (n=5). Significance was determined by two-way ANOVA with Bonferroni's correction (post tests). $^{###}$ and $^{\#}$ indicate P<0.001 and P<0.05, respectively, when compared to wild-type flies w^{1118}.

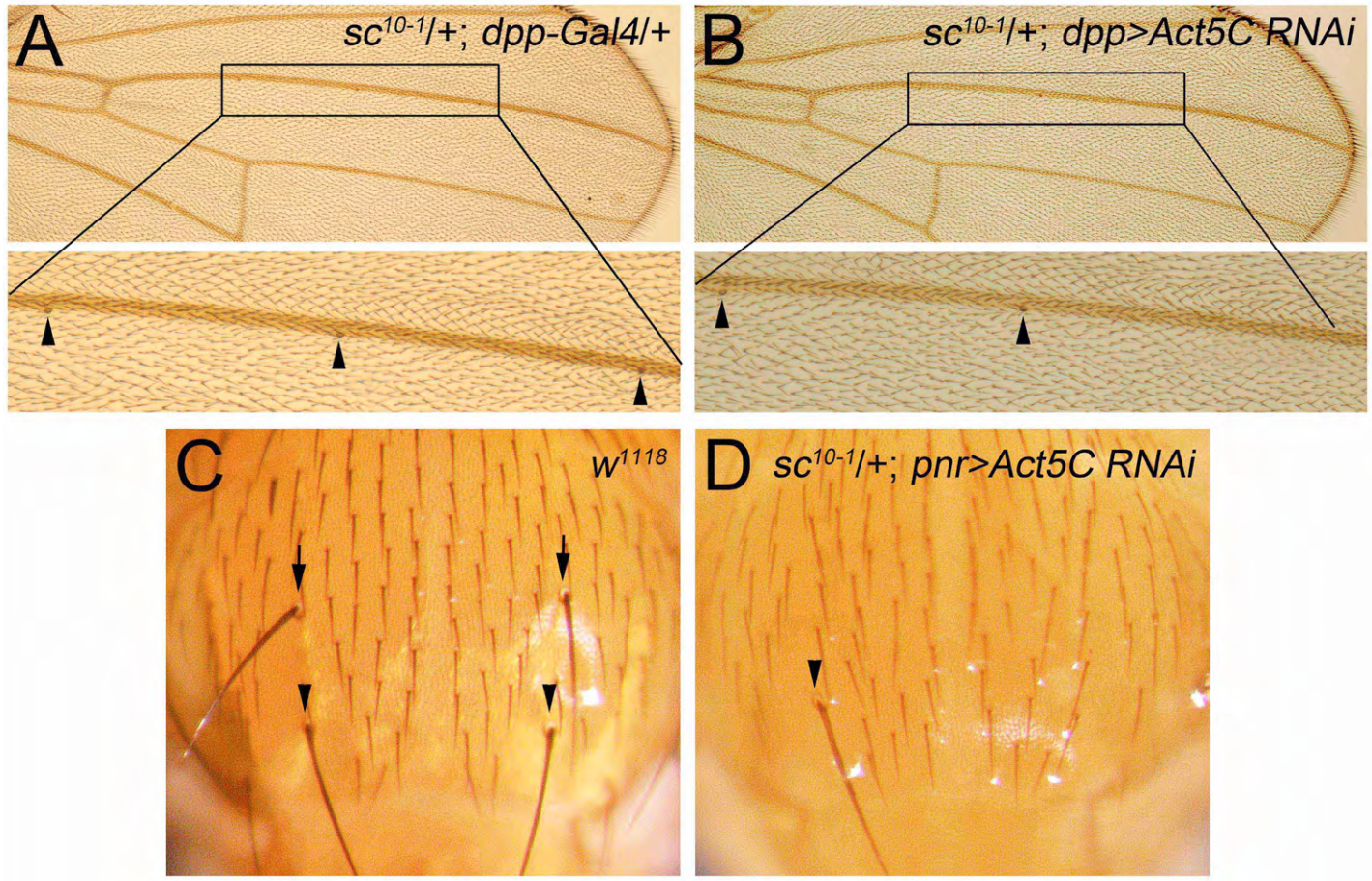

Fig. S2. Knockdown of *Act5C* by RNAi disrupts bristle formation in *ac sc* heterozygous flies. (A and B) Adult wings. Arrowheads indicate the campaniform sensilla on the LIII vein. Less than three campaniform sensilla were present on LIII vein of *sc*$^{10\text{-}1}$/+; *dpp >Act5C RNAi* wing. (C and D) Adult nota. Arrows and arrowheads in (C) indicate the anterior and posterior DC bristles, respectively, in wild-type notum. Some DC bristles were missing in *sc*$^{10\text{-}1}$/+; *pnr >Act5C RNAi* notum (D).

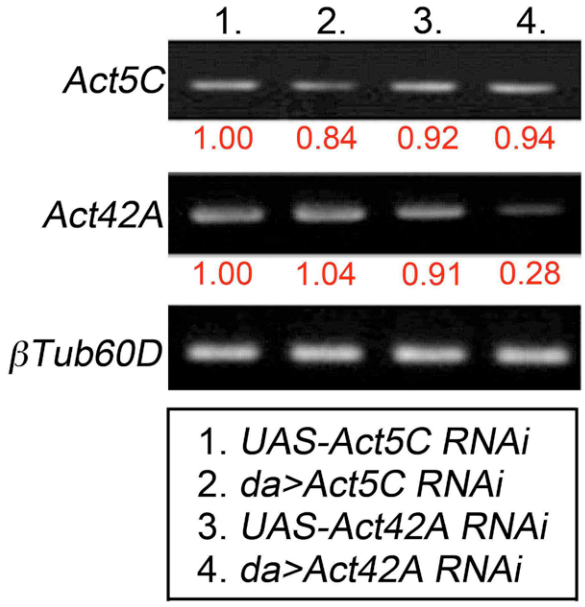

Fig. S3. Knockdown efficiency of *Act5C* RNAi and *Act42A* RNAi. RT-PCR analysis to show the levels of *Act5C* and *Act42A* mRNA. *βTub60D* was used as the loading control. Representative results from two independent experiments are shown. The numbers indicate the relative levels of RT-PCR products (*actin*/*βTub60D*) determined by DNA intensity analyzed by Image J.

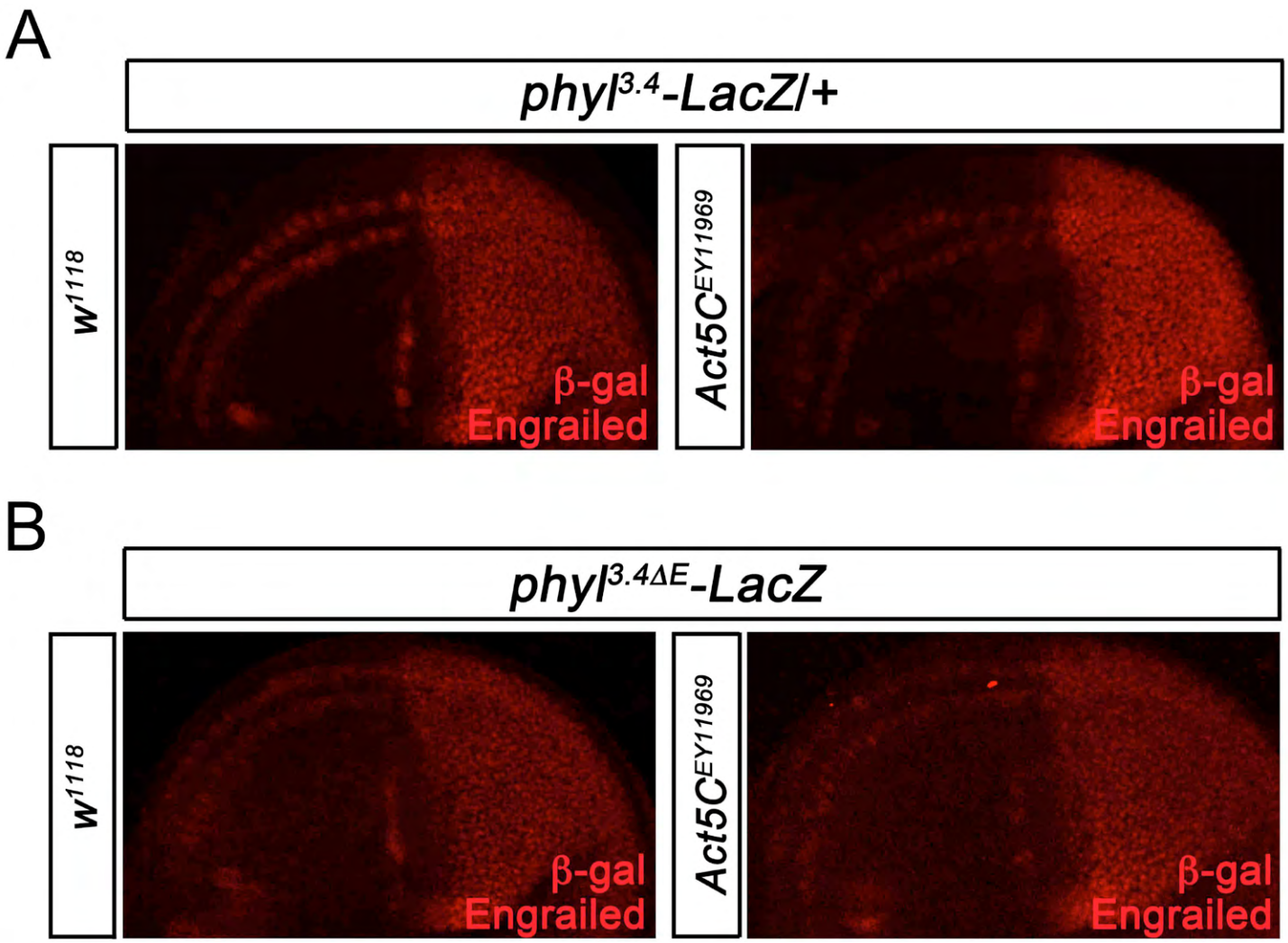

Fig. S4. *Act5C* regulates *phyllopod* expression in an E-box dependent manner. (A and B) Images of 0–1 hr APF wing discs from *phyl*$^{3.4}$*-LacZ*/+ (A) or *phyl*$^{3.4\Delta E}$*-LacZ* pupae (B), immunostained for β-galactosidase (red) and Engrailed (red). Anterior of the discs is to the left.

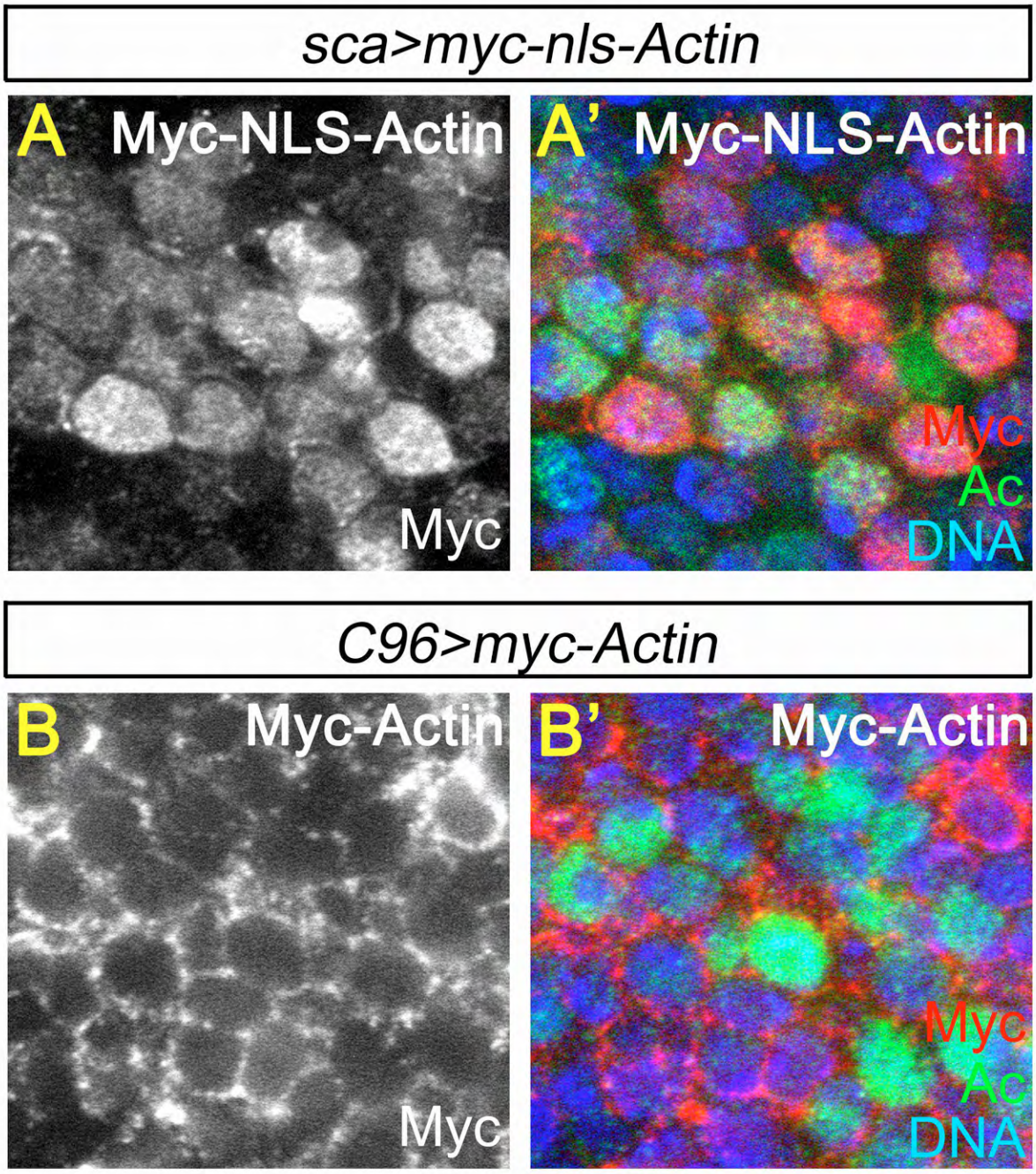

Fig. S5. NLS-actin localizes in nuclei of proneural cells. (A–B′) Wing discs immunostained with anti-Myc antibody (red), co-stained with anti-Ac antibody (green) and DNA dye Hoechst 33342 (blue). (A and A′) Myc-NLS-Actin accumulated in nuclei of Ac-positive proneural cells. (B and B′) Myc-Actin primarily localized in cytoplasm of Ac-positive proneural cells. Low levels of Myc-Actin were also detected in nuclei.

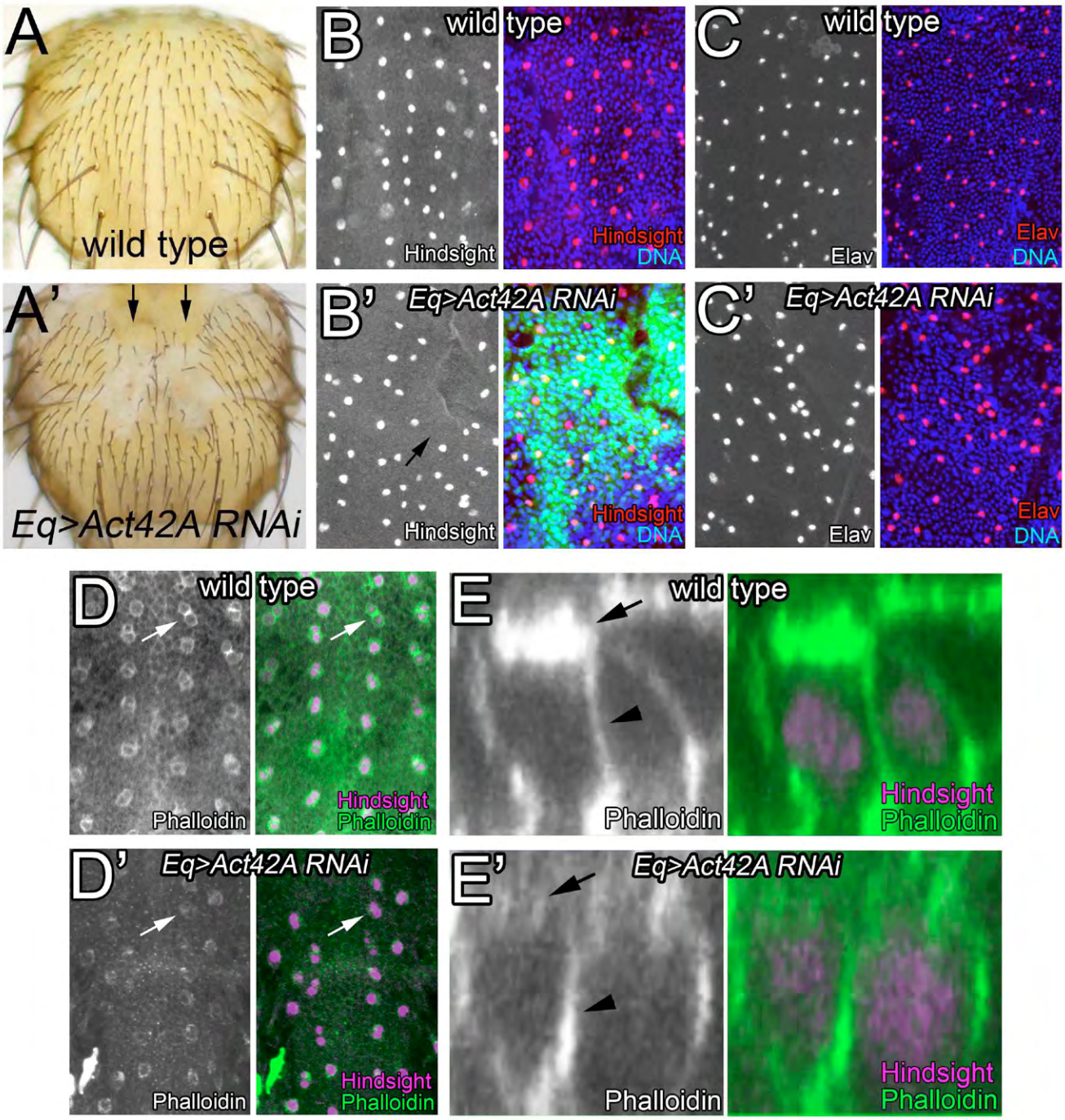

Fig. S6. Reduction of actin levels does not influence pIIa/pIIb cell fate determination during ES organ development. (A) Adult nota of wild-type and *Eq >Act42A* flies. Arrows in (A′) indicate where microchaetal bristles are missing. (B) Pupal nota immunostained with anti-Hindsight antibody, co-stained with anti-GFP antibody to mark the area where *Eq-Gal4* is expressed. Arrows in (B′) indicate where SOP specification is disrupted. (C) Pupa nota immunostained with anti-Elav antibody. Single Elav-positive cells observed in developing ES organs in both wild-type and *Eq >Act42A RNAi* nota. (D) Pupa nota immunostained with phalloidin. Phalloidin intensities in ARS (arrows) as well as in epithelium reduced in *Eq >Act42A RNAi* notum compared to wild-type notum. (E) Orthogonal sections showing ARS in two-cell cluster marked by anti-Hindsight staining (magenta). While the wild-type ARS has an umbrella-shaped structure with a lateral stalk (arrowhead) and an apical area (arrow) (E), the apical area of ARS in *Eq >Act42A RNAi* notum was reduced (E′).

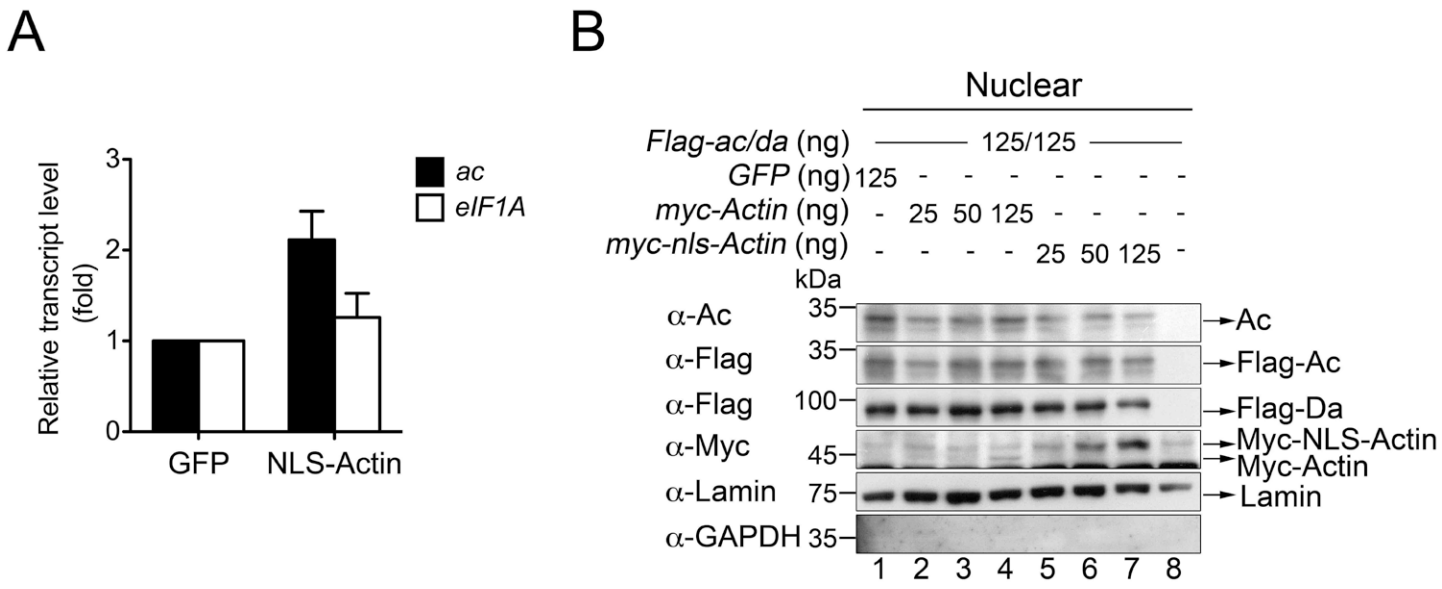

Fig. S7. Total nuclear Ac levels unaffected by NLS-Actin in S2 cells transfected with *ac* and *da*. (A) qRT-PCR analysis of endogenous *ac* and *eIF1A* mRNA in *ac* and *da*-transfected S2 cells, co-transfected with *GFP* or *myc-nls-Actin*. Transcript levels were normalized with that of *rpL32*. Average (mean ± SEM) is shown (*n*=3). (B) Western blot analysis showing comparable levels of total nuclear Ac detected by anti-Ac antibody in cells transfected with *GFP*, *myc-Actin* or *myc-nls-Actin*.

	Position	Sequence
Actin5C/Actin42A	20-29	AGFAGDDAPR
	30-40	AVFPSIVGRPR
	41-51	HQGVMVGMGQK
	52-63	DSYVGDEAQSKR
	86-96	IWHHTFYNELR
	97-114	VAPEEHPVLLTEAPLNPK
	179-192	LDLAGRDLTDYLMK
	198-211	GYSFTTTAEREIVR
	240-255	SYELPDGQVITIGNER
	292-313	KDLYANTVLSGGTTMYPGIADR
	361-373	QEYDESGPSIVHR
Actin5C	328-336	IKIIAPPER
Actin42A	328-336	IKIVAPPER

Table S1. Identified peptides from mass spectrometric analysis of the 42 kDa protein band. Peptides matching with either Act5C or Act42A are shown.