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Figures and figure supplements

Salt-inducible kinases mediate nutrient-sensing to link dietary sugar and tumorigenesis in *Drosophila*

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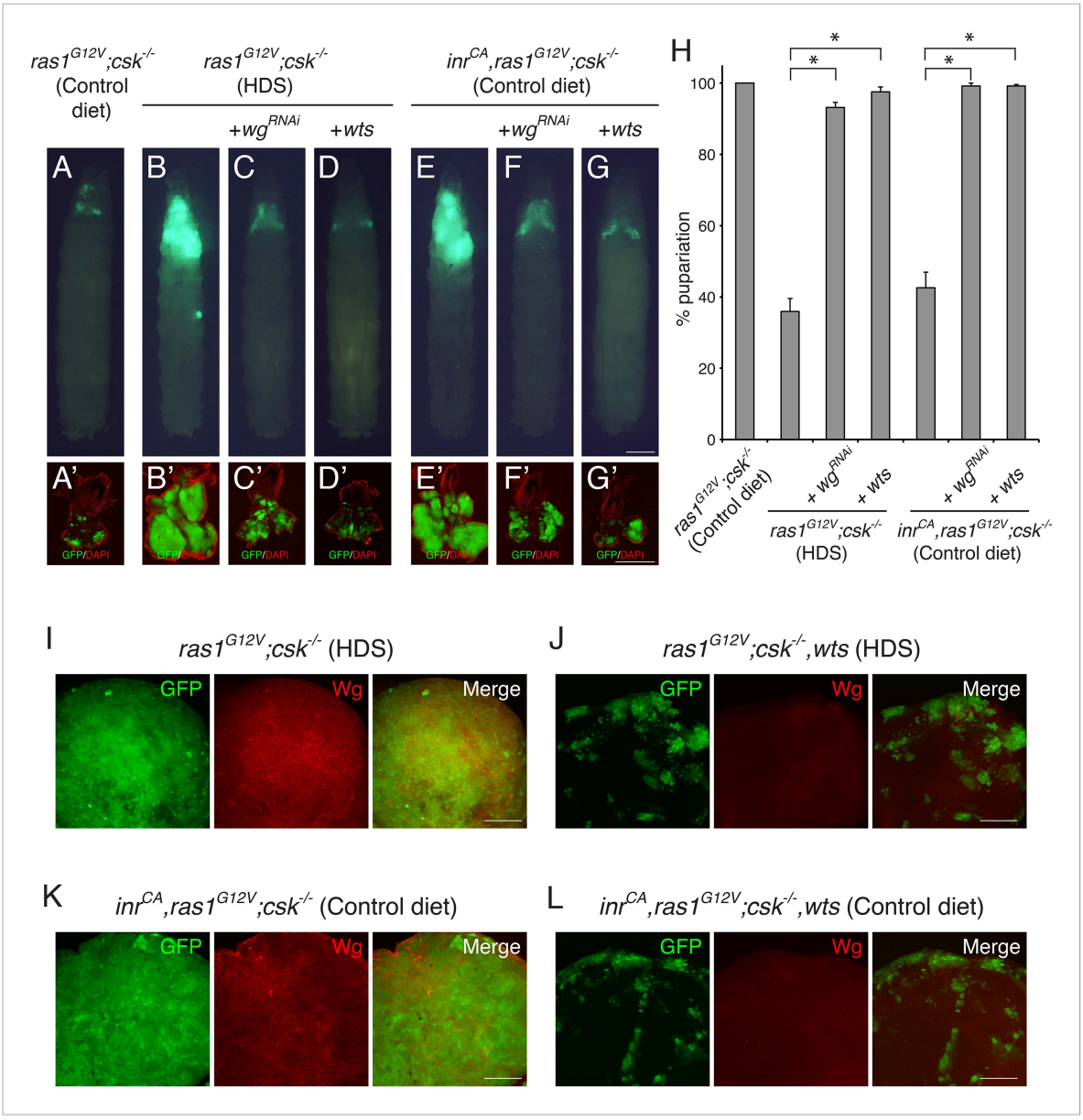

Figure 1. Yorkie Activity is Required for Increased Wg Expression in Diet-enhanced Ras/Src-tumors. (**A**–**G**) Developmental stage matched third instar larvae with the genotype, (**A**, **B**) $ras1^{G12V};csk^{-/-}$, (**C**) $ras1^{G12V};csk^{-/-},wg^{RNAi}$, (**D**) $ras1^{G12V};csk^{-/-},wts$, (**E**) $inr^{CA},ras1^{G12V};csk^{-/-}$, (**F**) $inr^{CA},ras1^{G12V};csk^{-/-},wg^{RNAi}$, and (**G**) $inr^{CA},ras1^{G12V};csk^{-/-},wts$, raised on indicated diets. Images were taken at the same magnification. Scale bar, 500 μm. (**A′**–**G′**) Matching dissected eye epithelial tissue stained with DAPI (red). Images were taken at the same magnification. Scale bar, 500 μm. (**H**) Percent pupariation of animals from indicated genotypes and diets. Column bars represent the mean of three independent experiments. Error bars denote s.e.m. Total *n* was 166, 431, 309, 291, 204, 200, and 251 from left to right. Asterisks indicate statistically significant difference (*p < 0.01 t-test). Numerical data are available in ***Figure 1—source data 1***. (**I**–**L**) Wg staining (red) of eye tissue from (**I**) $ras1^{G12V};csk^{-/-}$, (**J**) $ras1^{G12V};csk^{-/-},wts$, (**K**) $inr^{CA},ras1^{G12V};csk^{-/-}$, and (**L**) $inr^{CA},ras1^{G12V};csk^{-/-},wts$ animals raised on indicated diets. Scale bars, 50 μm.

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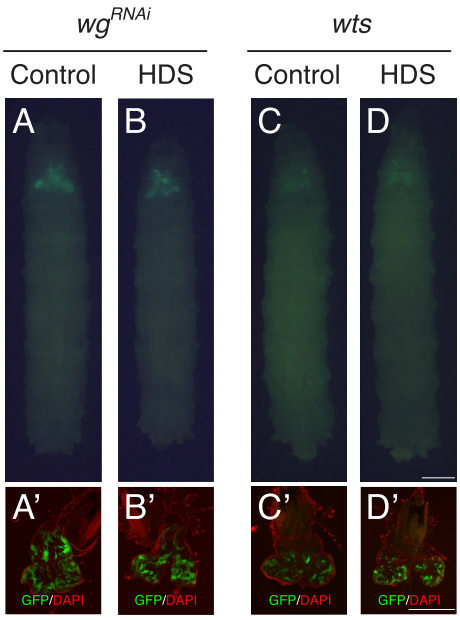

Figure 1—figure supplement 1. Effect of reducing Wg or over-expressing Wts in the eye tissue. (**A**, **B**) Reducing Wg by RNAi (wg^{RNAi}) did not affect normal eye tissue growth of the late third instar larvae. Developmental stage matched wg^{RNAi} third instar larvae raised on (**A**) control diet, and (**B**) HDS. (**C**, **D**) Over-expression of Warts kinase led to small clones. Developmental stage matched *wts* third instar larvae raised on (**C**) control diet, and (**D**) HDS. Images were taken at the same magnification. Scale bar, 500 μm. (**A'**–**D'**) Matching dissected eye epithelial tissue stained with DAPI (red). Images were taken at the same magnification. Scale bar, 500 μm.

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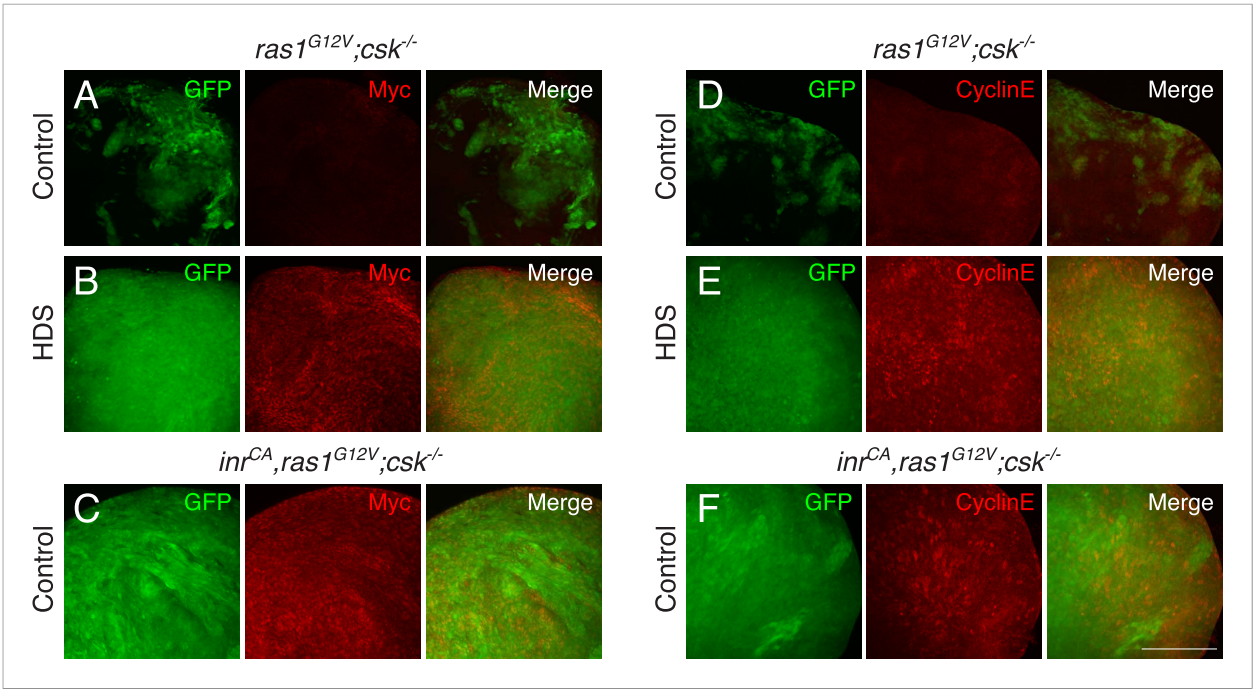

Figure 1—figure supplement 2. Yorkie target genes are upregulated in diet-enhanced Ras/Src-tumors. (**A**–**C**) Myc staining (red) of (**A**) *ras1^{G12V};csk$^{-/-}$* in control diet, (**B**) *ras1^{G12V};csk$^{-/-}$* in HDS, and (**C**) *inr^{CA},ras1^{G12V};csk$^{-/-}$* in control diet. (**D**–**F**) Cyclin E staining (red) of (**D**) *ras1^{G12V};csk$^{-/-}$* in control diet, (**E**) *ras1^{G12V};csk$^{-/-}$* in HDS, and (**F**) *inr^{CA},ras1^{G12V};csk$^{-/-}$* in control diet. Images were taken at the same magnification. Scale bar, 50 μm.
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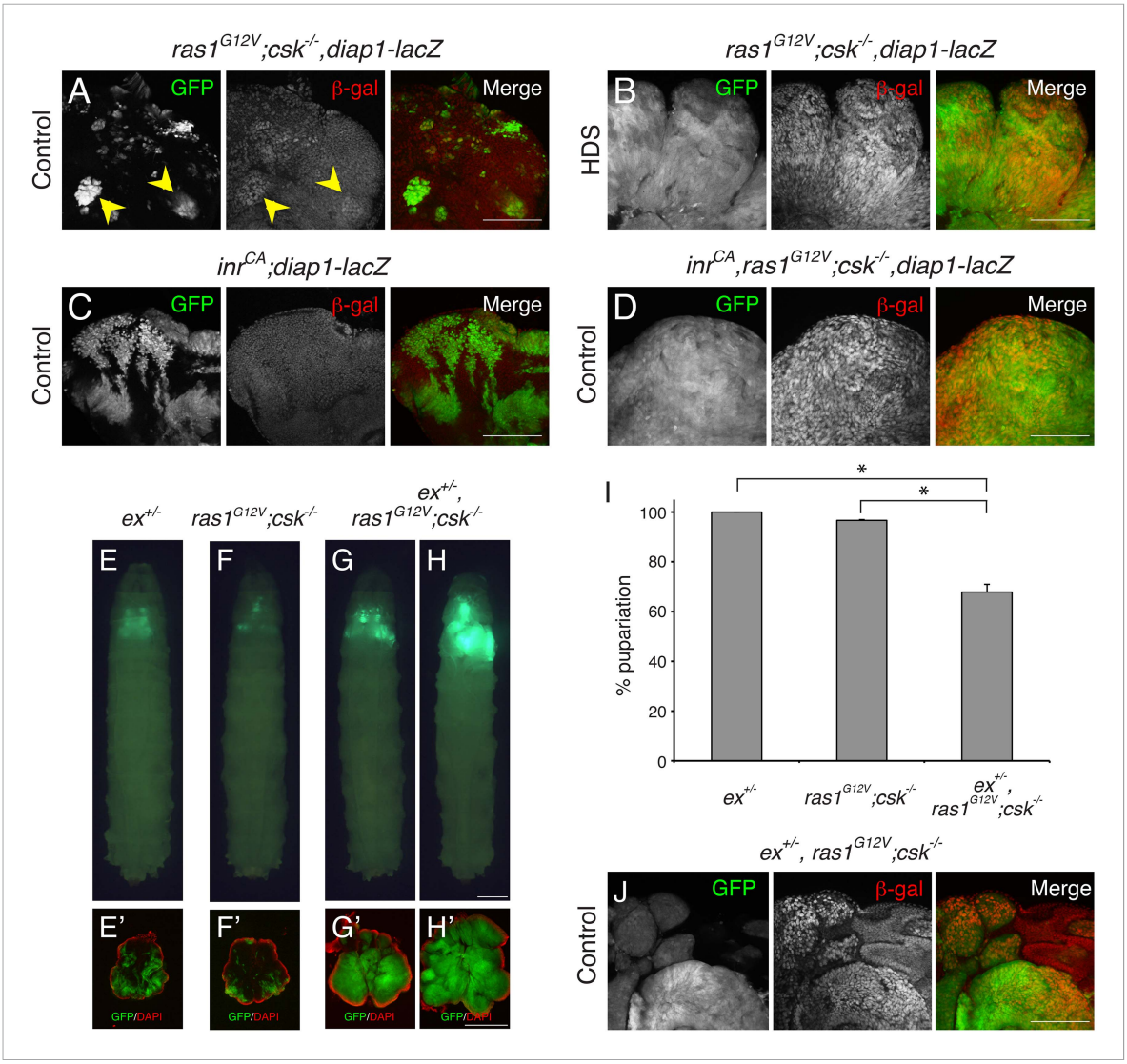

Figure 2. Ras/Src-activated Cells are Sensitive to Perturbations in the Hippo Signaling. (**A**–**D**) β-galactosidase (β-gal) staining (red) of eye tissue from (**A**, **B**) *ras1*^{G12V};*csk*$^{-/-}$,*diap1-lacZ*, (**C**) *inr*^{CA};*diap1-lacZ*, (**D**) *inr*^{CA},*ras1*^{G12V};*csk*$^{-/-}$,*diap1-lacZ* animals raised on indicated diets. Scale bars, 50 μm. (**E**–**H**) Developmental stage matched third instar larvae raised on control diet with the genotype, (**E**) *ex*$^{+/-}$, (**F**) *ras1*^{G12V};*csk*$^{-/-}$, (**G**, **H**) *ex*$^{+/-}$,*ras1*^{G12V};*csk*$^{-/-}$. Images were taken at the same magnification. Scale bar, 500 μm. (**E′**–**H′**) Matching dissected eye epithelial tissue stained with DAPI (red). Images were taken at the same magnification. Scale bar, 500 μm. (**I**) Percent pupariation of animals from indicated genotypes. Column bars represent the mean of three independent experiments. Error bars denote s.e.m. Total *n* of 389, 238, and 206 from left to right. Asterisks indicate statistically significant difference (*p < 0.01 t-test). Numerical data are available in ***Figure 2—source data 1*** (**J**) β-galactosidase (β-gal) staining (red) of *ex*$^{+/-}$, *ras1*^{G12V};*csk*$^{-/-}$ animals raised on control diet. Scale bar, 50 μm.

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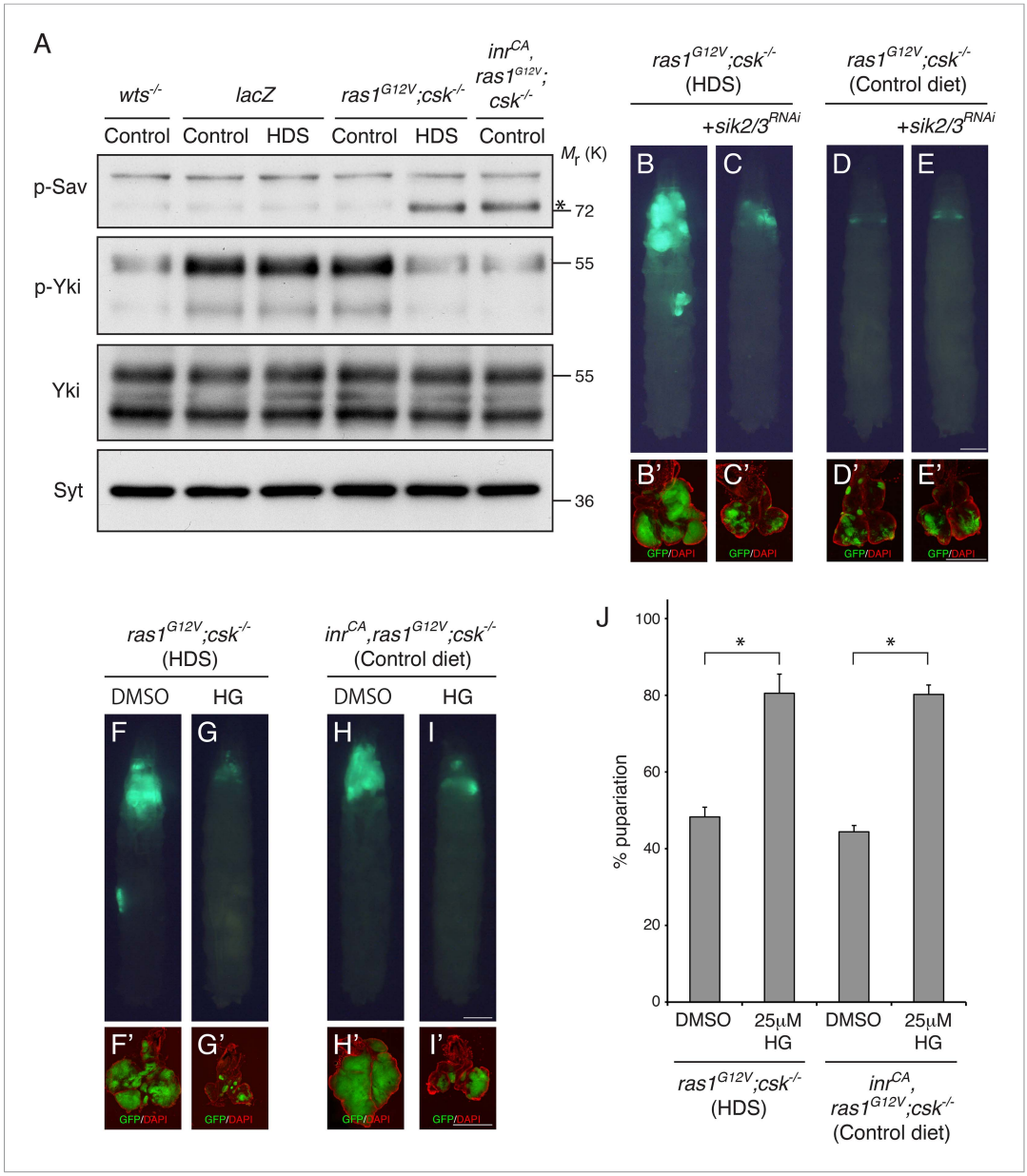

Figure 3. Salt-inducible Kinases are Required for Diet-enhanced Ras/Src-tumorigenesis. (**A**) Extracts from dissected eye tissues of third instar larvae were examined by immunoblotting using antibodies against phospho-Sav (p-Sav; * indicates p-Sav specific band; the upper band is a non-specific band showed as an internal loading control), phospho-Yki (p-Yki), total Yki (Yki), and Syntaxin (Syt). (**B**, **C**) Developmental stage matched third instar larvae raised on HDS with the genotype, (**B**) $ras1^{G12V};csk^{-/-}$, and (**C**) $sik2/3^{RNAi},ras1^{G12V};csk^{-/-}$. (**D**, **E**) Developmental stage matched third instar larvae raised on control diet with the genotype, (**D**) $ras1^{G12V};csk^{-/-}$, and (**E**) $sik2/3^{RNAi},ras1^{G12V};csk^{-/-}$. Images were taken at the same magnification. Scale bar, 500 μm. (**F**, **G**) $ras1^{G12V};csk^{-/-}$ animals raised on HDS containing (**F**) 0.05% DMSO, or (**G**) 25 μM HG-9-91-01. (**H**, **I**) $inr^{CA},ras1^{G12V};csk^{-/-}$ animals raised on control diet containing (**H**) 0.05% DMSO, or (**I**) 25 μM HG-9-91-01. Images were taken at the same magnification. Scale bar, 500 μm. (**B′**–**I′**) Matching dissected eye epithelial tissue stained with DAPI (red). Images were taken at the same magnification. Scale bar, 500 μm. (**J**) Percent pupariation of DMSO or HG-9-91-01 treated animals from indicated genotypes and diets. Column bars represent the mean of three independent experiments. Error bars denote s.e.m. Total *n* of 139, 76, 123, and 72 from left to right. Asterisks indicate statistically significant difference (*$p < 0.01$ t-test). Numerical data are available in ***Figure 3—source data 1***.

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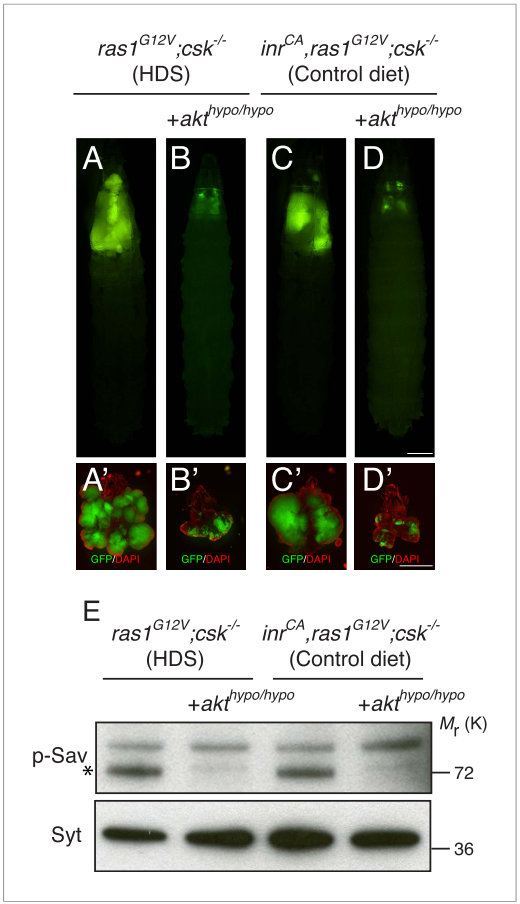

Figure 3—figure supplement 1. Akt mediates activation of SIKs in Ras/Src-tumors. (**A**, **B**) Developmental stage matched third instar larvae raised on HDS with the genotype, (**A**) *ras1*^{G12V};*csk*$^{-/-}$, and (**B**) *ras1*^{G12V};*csk*$^{-/-}$,*akt*$^{hypo/hypo}$. (**C**, **D**) Developmental stage matched third instar larvae raised on control diet with the genotype, (**C**) *inr*^{CA},*ras1*^{G12V};*csk*$^{-/-}$, and (**D**) *inr*^{CA},*ras1*^{G12V};*csk*$^{-/-}$,*akt*$^{hypo/hypo}$. Images were taken at the same magnification. Scale bar, 500 μm. (**A**′–**D**′) Matching dissected eye epithelial tissue stained with DAPI (red). Images were taken at the same magnification. Scale bar, 500 μm. (**E**) Extracts from dissected eye tissues of third instar larvae were examined by immunoblotting using antibodies against phospho-Sav (p-Sav; * indicates p-Sav specific band; the upper band is a non-specific band showed as an internal loading control) and Syntaxin (Syt).

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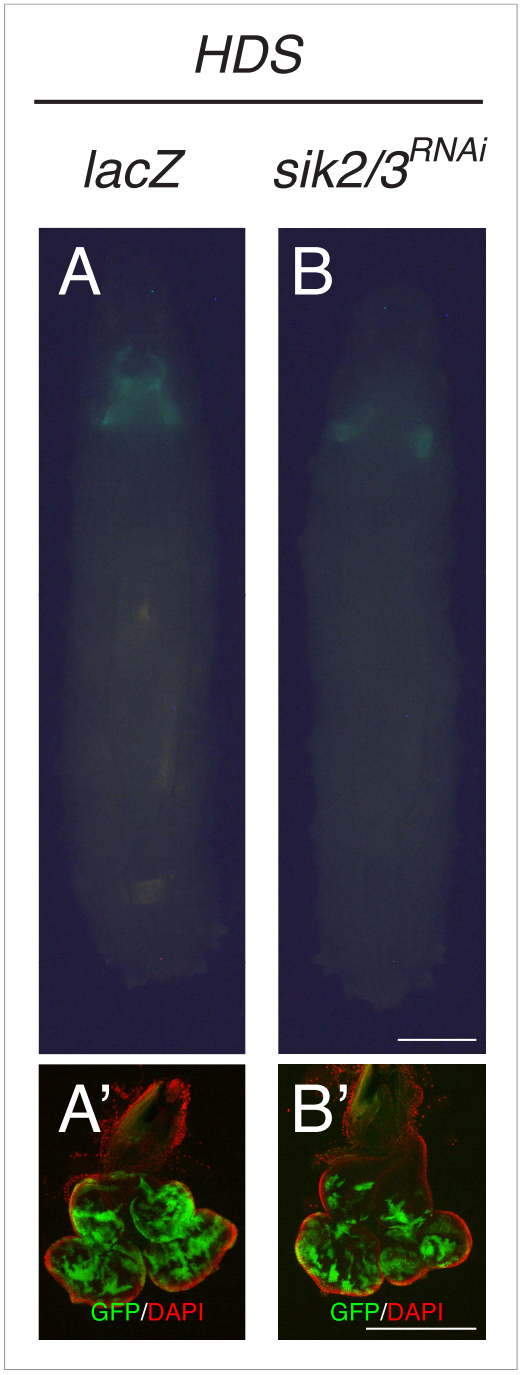

Figure 3—figure supplement 2. Reducing SIK2/3 by RNAi did not affect normal eye tissue growth. (**A**, **B**) Developmental stage matched third instar larvae raised on HDS with genotypes, (**A**) *lacZ*, and (**B**) *sik2/3*^{RNAi}. Images were taken at the same magnification. Scale bar, 500 μm. (**A′**, **B′**) Matching dissected eye epithelial tissue stained with DAPI (red). Images were taken at the same magnification. Scale bar, 500 μm.

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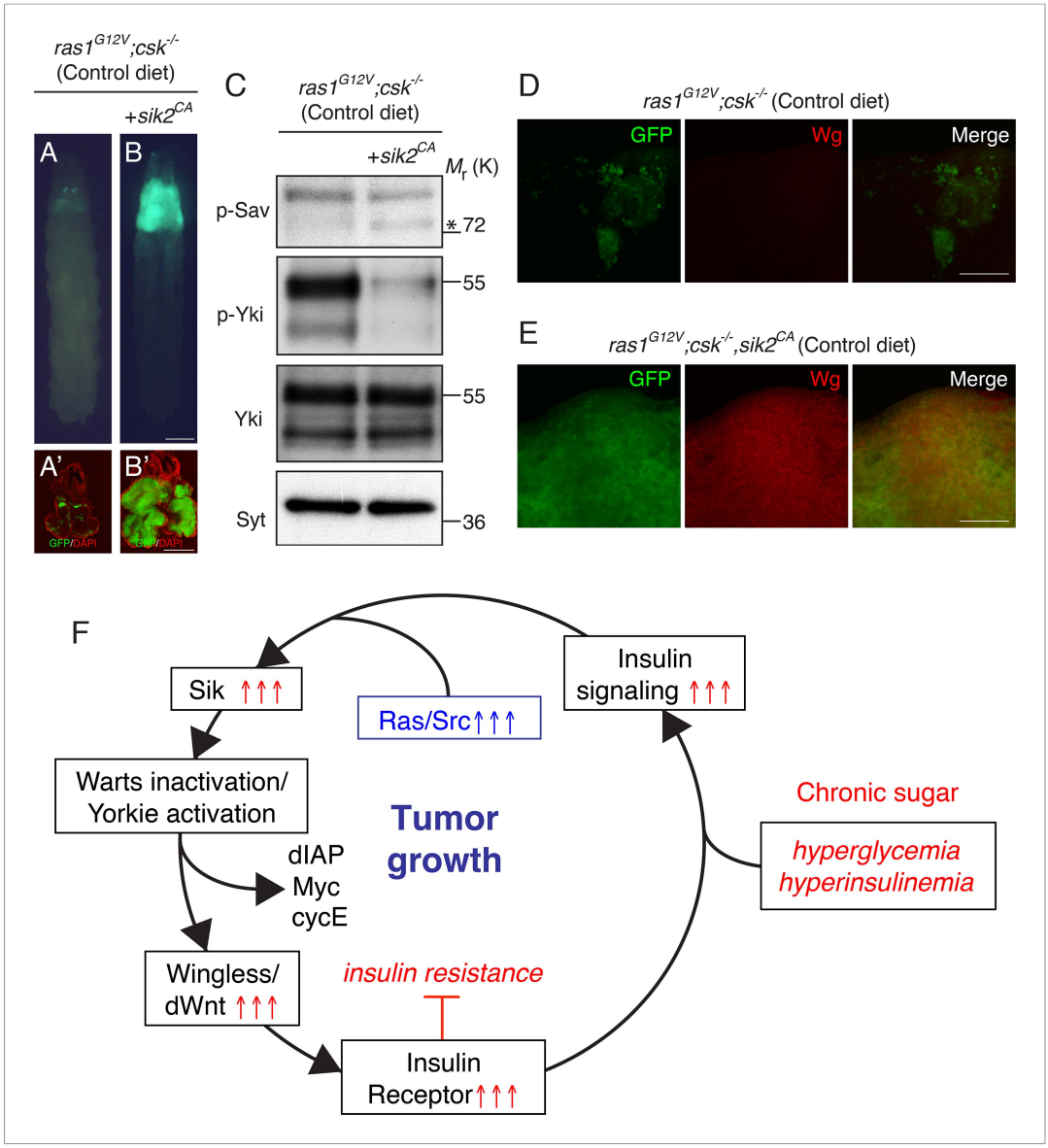

Figure 4. Activation of Salt-inducible Kinase Promotes Ras/Src-tumor Growth. (**A**, **B**) Developmental stage matched third instar larvae raised on control diet with the genotype, (**A**) $ras1^{G12V};csk^{-/-}$, and (**B**) $ras1^{G12V};csk^{-/-},sik2^{CA}$. Images were taken at the same magnification. Scale bar, 500 μm. (**A′**, **B′**) Matching dissected eye epithelial tissue stained with DAPI (red). Images were taken at the same magnification. Scale bar, 500 μm. (**C**) Extracts from dissected eye tissues of $ras1^{G12V};csk^{-/-}$ and $ras1^{G12V};csk^{-/-},sik2^{CA}$ animals fed a control diet were examined by immunoblotting using antibodies against phospho-Sav (p-Sav; * indicates p-Sav specific band; the upper band is a non-specific band showed as an internal loading control), phospho-Yki (p-Yki), total Yki (Yki), and Syntaxin (Syt). (**D**, **E**) Wg staining (red) of eye tissue from (**D**) $ras1^{G12V};csk^{-/-}$, and (**E**) $ras1^{G12V};csk^{-/-},sik2^{CA}$ animals raised on control diet. Scale bars, 50 μm. (**F**) Model of diet-enhanced tumorigenesis of Ras/Src-activated cells.

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