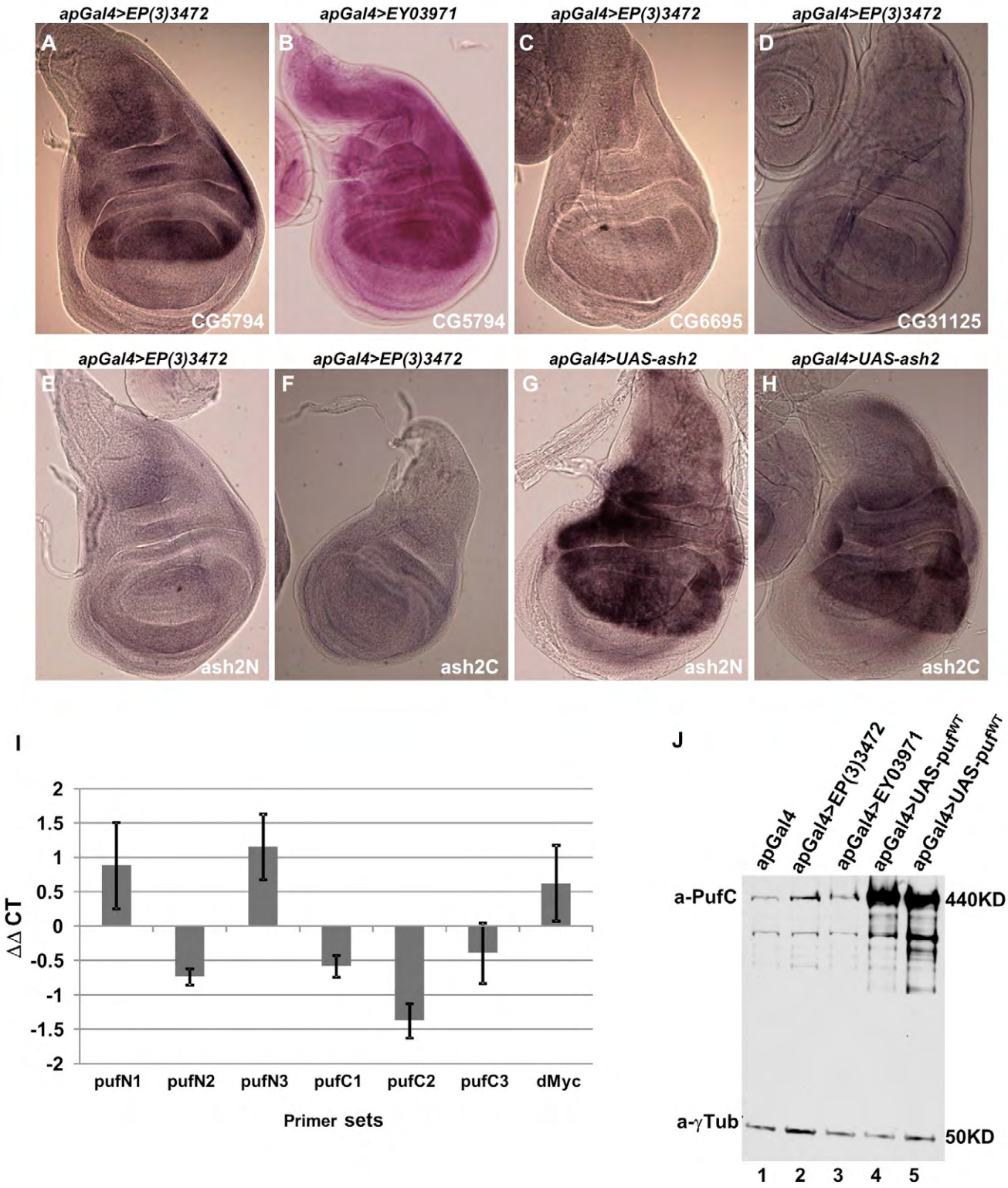

Fig. S1. Activation of *EP(3)3472* and *EY03971* induces *CG5794* expression. (A-F) In situ hybridizations of 3rd instar larval wing discs overexpressing *EP(3)3472* or *EY03971* show only CG5794 expression was induced. (A) *apGal4*; *EP(3)3472* and (B) *apGal4*; *EY03971* induced CG5794 expression. *apGal4*; *EP(3)3472* did not induce CG6695 (C) or CG31125 (D) expression. *apGal4*; *EP(3)3472* did not induce ash2 expression as indicated by anti-sense RNA probe against N-terminal (E) or C-terminal of ash2. (G,H) *apGal4*; *UAS-ash2* activated ash2 expression. (I)). qRT-PCR showing detection of puf transcripts in puf mutant (puf^{A495}) using various primers, and puf mutant has no significant effect on dMyc transcript levels. 30 wing discs per genotype were used to isolate mRNA. Relative expression levels (ΔΔCT) were calculated using *RpS16* as internal control. Data represent mean of three biological samples analyzed in duplicate. Error bars reflect standard error of the mean. Transgenes were induced for 20h using temperature inducible Gal4 drivers. (J) Western blot showing *UAS-puf* expressed (lane 4-5) Puf at much higher level than w^{1118} control (lane1), *EP(3)3472* (lane 2) or *EY03971*(lane3). Each lane used protein lysates from 10 wing discs.

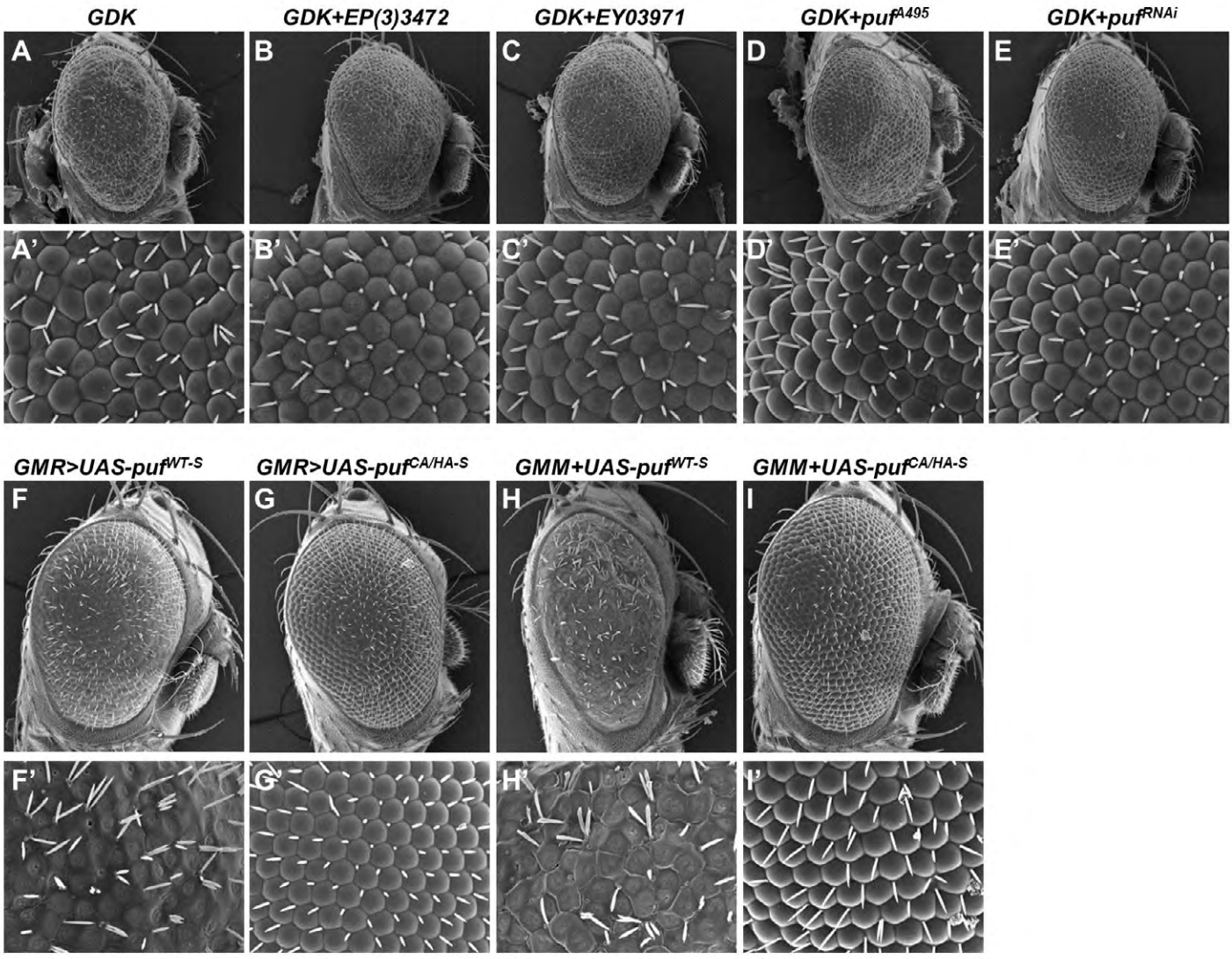

Fig. S2. Puf does not modify GDK dependent eye phenotype. (A-E) Scanning electron micrographs (SEM) of adult compound eyes show that Puf does not modify *GDK (GMR-Gal4, UAS-cycD, UAS-ckd4*) eye phenotype. (F-I) SEM of adult compound eye show that short isoform of Puf functions similarly to the long isoform. (A, A') *GDK*; (B,B') *GDK, EP(3)3472/+*; (C,C') *GDK, EY03971*/+; (D,D') *GDK*, puf^{A459}/+; (E,E') *GDK,UAS-pufRNAi*/+; (F,F') *GMR-Gal4, UAS-*puf^{WTS}/+; (G,G') *GMR-Gal4,UAS-*$puf^{CA/HAS}$/+; (H,H') *GMM,UAS-*puf^{WTS}/+; (I,I) *GMM, UAS-*$puf^{CA/HAS}$/+. SEM magnification for (A-I) is 160x and 750x for (A'-I')

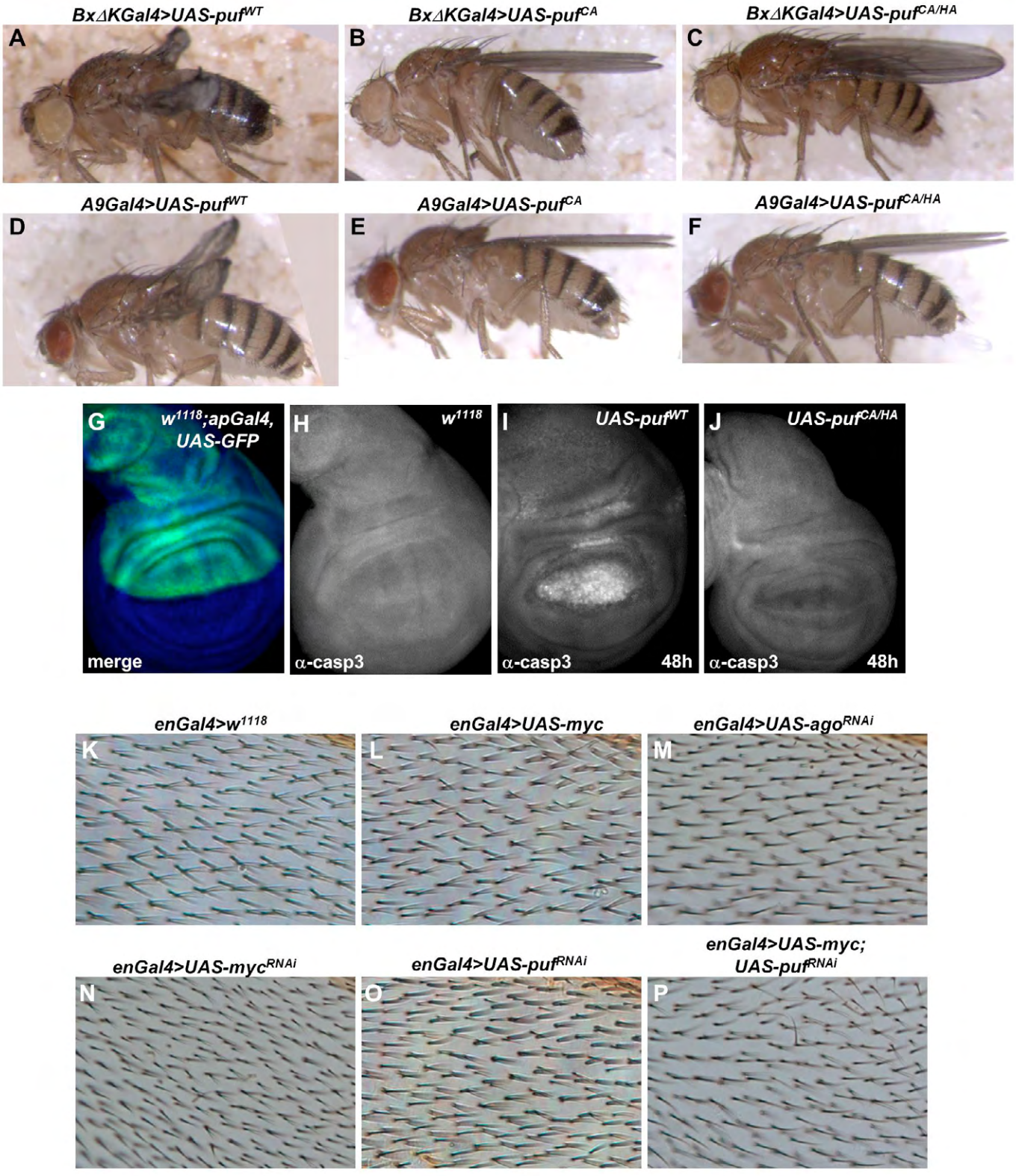

Fig. S3. Puf is necessary for cell growth in the wing. (A-F) *UAS-puf* induced wing phenotype depends on its catalytic activity. (G-J) *UAS-puf* induced apoptosis in an enzymatic dependent manner 48h after induction as indicated by cleaved caspase 3 (casp3) staining. (K-P) Reduced dMyc or Puf levels resulted in smaller cell size as demonstrated by increased density of the bristles. (A*) BxDKGal4, UAS-puf*^{WT}; (B) *BxDKGal4,UAS-puf*^{CA}; (C) *BxDKGal4,UAS-puf*$^{CA/HA}$; (D) *A9Gal4,UAS-puf*^{WT}; (E*) A9-Gal4,UAS-puf*^{CA}; (F) *A9Gal4,UAS-puf*$^{CA/HA}$. (G,H) *w*^{1118}*, apGal4, UAS-GFP* control*;* (I) *apGal4,UAS-puf*^{WT}; (J) *apGal4,UAS-puf*$^{CA/HA}$; (K) *w*^{1118}*, enGal4* control; (L) *enGal4,UAS-dMyc*; (M) *enGal4,UAS-agoRNAi;* (N) *enGal4,UAS-MycRNAi*; (O) *enGal4,UAS-pufRNAi*(KK)*;* (P) *enGal4,UAS-dMyc,UAS-pufRNAi*.

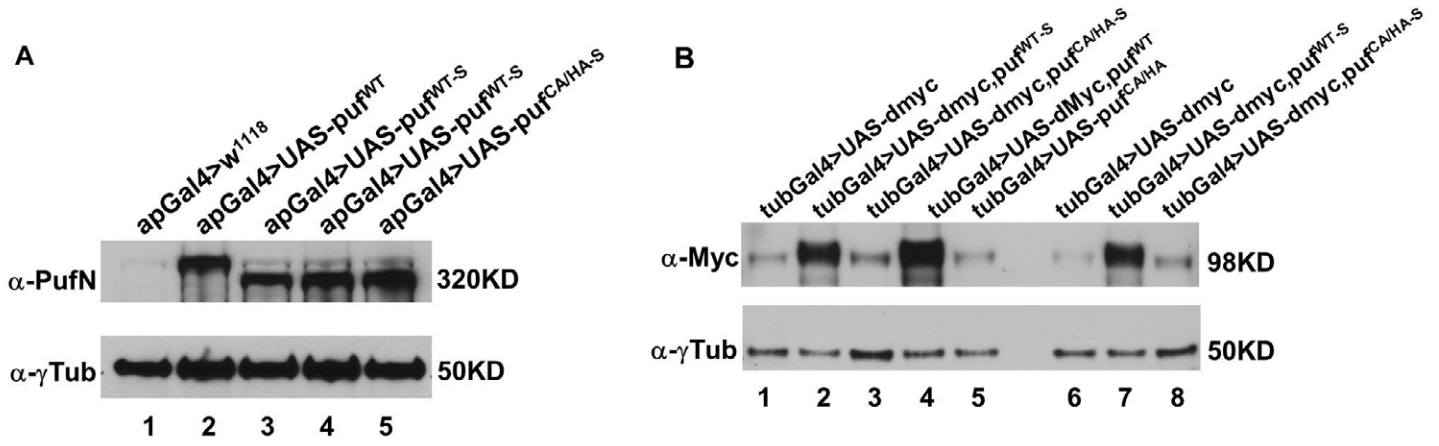

Fig. S4. Short isoform of Puf affects dMyc levels. (A) Western blot shows short isoform of Puf transgenes *UAS-puf*$^{WT-S}$ and *UAS-puf*$^{CA/HA-S}$ (lanes 3-5) expressed at similar levels as the long isoform *UAS-puf*^{WT}(lane 2). (B) Both long (lanes 4,5) and short isoforms (lane 2,3,7,8) of Puf regulate dMyc levels dependent on a WT catalytic domain. Protein lysates form 10 wing discs were used for each lane. All transgenes were induced for 18h using temperature inducible Gal4 drivers.

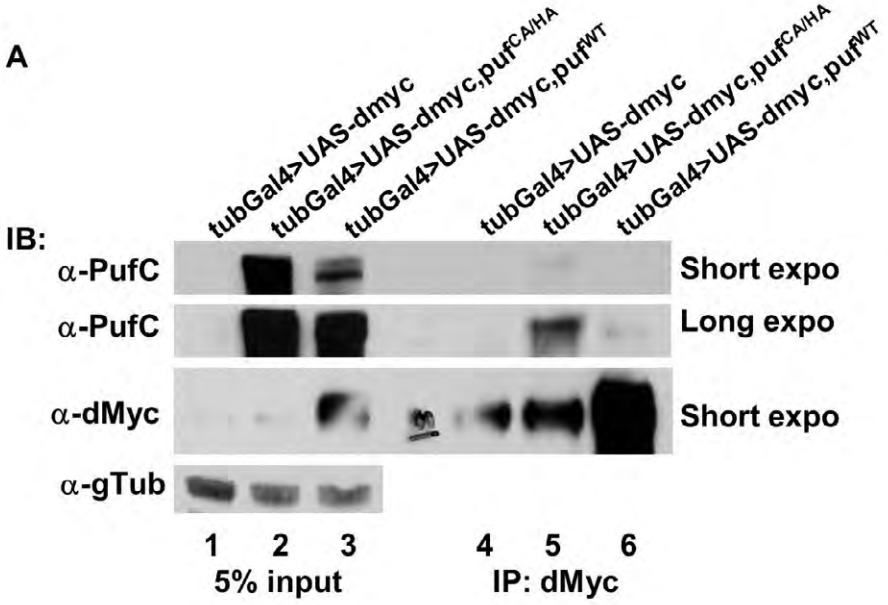

Fig. S5. dMyc and Puf form a protein complex. (A) Co-IP showing that both Puf^{WT} (lane 5) and Puf$^{CA/HA}$ (lane 6) form a protein complex with dMyc *in vivo*. (Lanes 1-3) Input for each genotype. Protein lysates isolated from wing discs of 3rd instar larvae overexpressing dMyc and dMyc+Puf^{WT} or dMyc+Puf$^{CA/HA}$ were immunoprecipitated with anti-dMyc antisera and analyzed by western blots using anti-PufC.

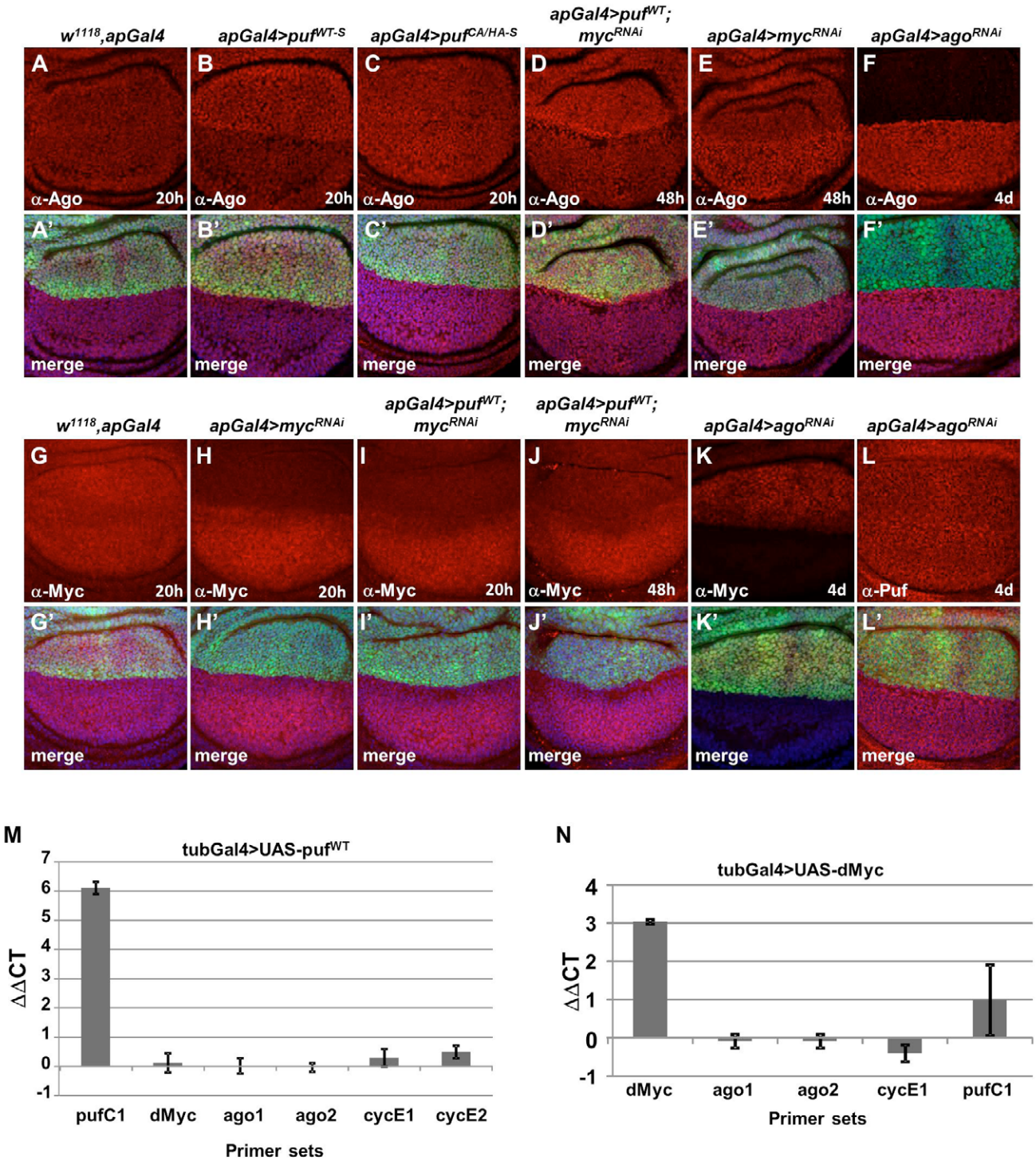

Fig. S6. Ago levels are regulated by Puf and dMyc. (A-C) Immunostaining of the 3rd instar larval wing disc 20 hours after induction showing Puf and dMyc regulate Ago levels. Transgene expression is marked by GFP. (A, A') *w*^{1118}*,ap-Gal4,UAS-GFP* serves as control showing Ago expression pattern. (B,B') effect of wildtype Puf short isoform (*ap-Gal4, UAS-GFP, UAS-puf*$^{WT\text{-}S}$) on endogenous Ago. (C,C') effect of short isoform of enzymatic inactive Puf (*ap-Gal4,UAS-GFP,UAS-puf*$^{CA/HA\text{-}S}$) on endogenous Ago. (D-E) Immunostaining of the 3rd instar larval wing disc showing Puf and dMyc regulate Ago levels 48 hours after induction of transgene. (D,D') Knockdown of *dmyc* (*ap-Gal4, UAS-GFP, UAS-dmyc*^{RNAi}); (E,E') induction of Puf in the presence of *dmyc* knockdown (*ap-Gal4, UAS-GFP, dmyc*^{RNAi}*,UAS-puf*^{WT}). (F,F) Immunostaining of the 3rd instar larval wing disc showing reduced Ago levels after *ago* RNAi knockdown (*ap-Gal4,UAS-GFP,UAS-ago*^{RNAi}). (G,G') *w*^{1118}*,ap-Gal4,UAS-GFP* serves as control showing *dmyc* expression pattern. (H-J) Effect of *dmyc* knockdown by induction of *dmyc*RNAi transgene at various time points (*ap-Gal4,UAS-GFP,UAS-dmyc*^{RNAi}). (K-L) Effect of *ago* knockdown (*ap-Gal4,UAS-GFP,UAS-ago*^{RNAi}) on endogenous dMyc (K.K') or Puf (L,L'). (M) qRT-PCR showing Puf overexpression has no effect on *dmyc*, *ago* and *cycE* transcript levels. (N) qRT-PCR showing dMyc overexpression has no effect on *ago*, *cycE* and *puf* transcript levels. Relative expression levels (ΔΔCT) were calculated using *RpS16* as internal control. Data represent mean of three biological samples analyzed in duplicate. Error bars reflect standard error of the mean. Transgenes were induced for 20h using temperature inducible Gal4 drivers.

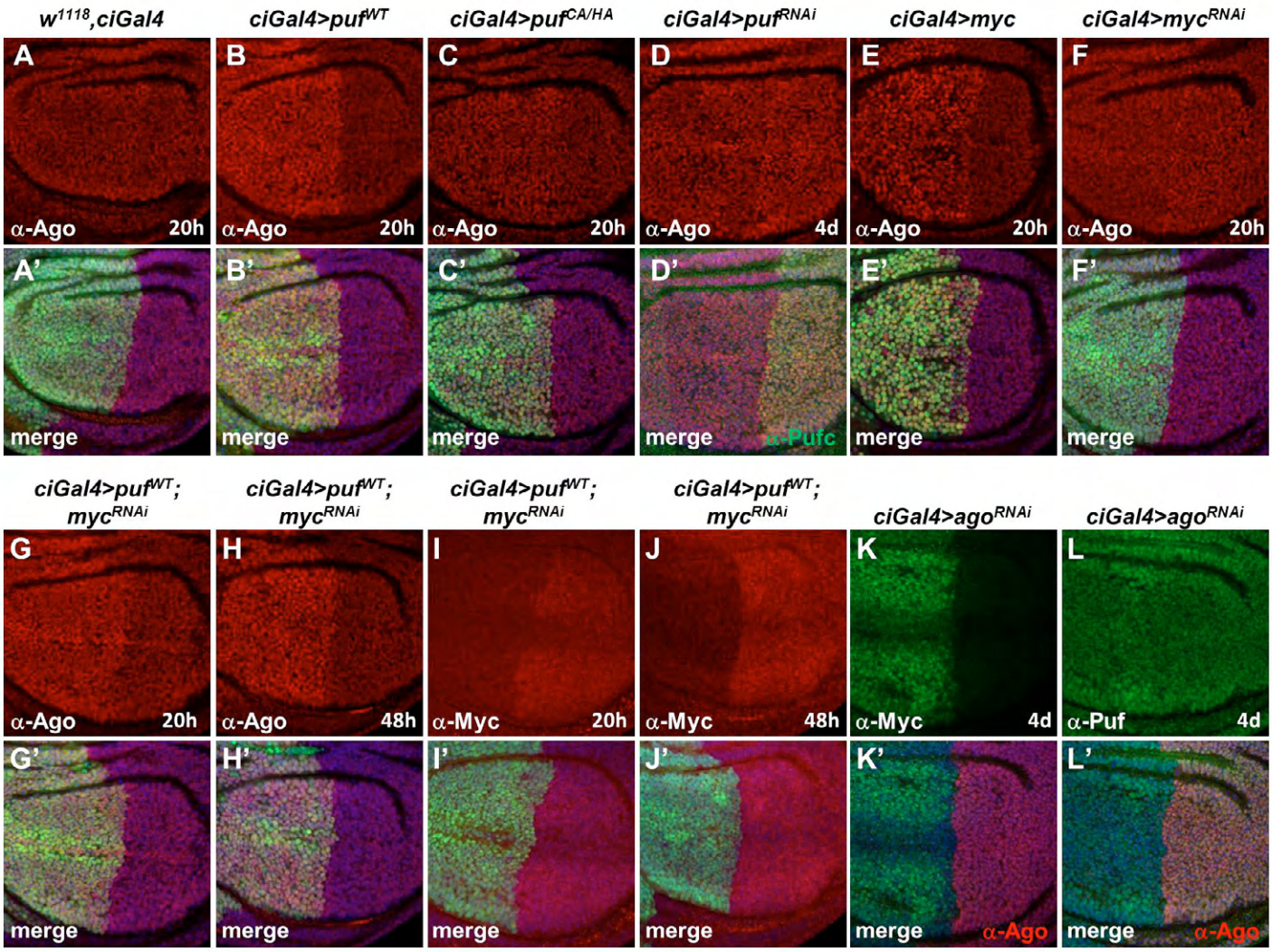

Fig. S7. Ago levels are regulated by Puf and dMyc. (A-H) Immunostaining of the 3rd instar larval wing disc showing Puf and dMyc regulate Ago levels. (A, A') *w*^{1118}*,ci-Gal4,UAS-GFP* serves as control showing Ago expression pattern. (B,B') effect of wildtype Puf (*ci-Gal4, UAS-GFP, UAS-puf*^{WT}) on endogenous Ago. (C,C') effect of enzymatic inactive Puf (*ci-Gal4,UAS-GFP,UAS-puf*$^{CA/HA}$) on endogenous Ago. (D,D') effect of Puf knockdown (marked by anti-PufC) on Ago (*ci-Gal4, UAS-puf*^{RNAi}). (E,E') effect of dMyc overexpression on Ago (*ci-Gal4, UAS-GFP, UAS-dmy)*. (F,F') effect of dMyc knockdown on Ago (*ci-Gal4, UAS-GFP, UAS-dmyc*^{RNAi}). (G-J) effect of Puf overexpression in the presence of dMyc knockdown (*ci-Gal4, UAS-GFP, UAS-puf*^{WT}*, dmyc*^{RNAi}) on Ago (G,H) and dMyc (I,J) at indicated time points. (K-L) effects of ago knockdown (*ci-Gal4,UAS-GFP,UAS-ago*^{RNAi}) on endogenous dMyc (K.K') or Puf (L,L').

Table S1. Primers used for cloning

Primers for *puf* wildtype cloning		
	Forward	**Reverse**
N-terminal	CATCCTAAATGGCATTGCAC	CCACATCCATCAGATCGACA
Mid 1	GCAAAAAGACGAGCAACAAG	CCACTACCGAAAGTGCTGGT
Mid 2	GTAAATCCCCAGCACCACAC	CTGCAGATGCTCTGGCAGTC
Mid 3	CACTTTTCCTTTCCGCTACG	ATCAGCGTGGACCAAGAGAC
C-terminal	CCCTCAATCCGCACAGTTAT	CTAAATCTGTGTTGGACTTGCCG
C-terminal NdeI site		CCATTATGAATCTGTGTTGGAC TTGCCGCCT

Primers for *puf* catalytic mutations		
	Forward	**Reverse**
Cys-Ala	CTAATTTGGGAGCCACTGCC TATATGGCCTCTTGCG	ACGCAAGAGGCCATATAGG CAGTGGCTCCCAAATTA
His 1-Ala	CTGGTGGGCGTCACTGTCGC CACGGGCACAGCGGATGG	CCATCCGCTGTGCCCGTG GCGACAGTGACGCCCACCAG
His 2-Ala	CGCCACGGGCACAGCGGC TGGCGGCGCCTACTACAGC TTTATAAAGG	CCTTTATAAAGCTGTAGT AGGCGCCGCCAGCCGCTG TGCCCGTGGCG

Table S2. Quantitative real-time PCR primers

Primer sequences from IDT (http://www.idtdna.com/site) were as follows.

	Primers for RT-PCR	
	Forward	Reverse
RpS16	CTGGAGCCAGTTCTGCTTCT	TCTCCTTCTTGGAAGCCTCA
CamKII	AAGCAAGGACATGCACATACC	GCAGATGCACTTCGATGAAA
pufN1	CAAGAGCCTGGTCGACTTCT	CATCTTCTCCACGGACAGC
pufN2	AACCAAATGGTGCGTCAAAT	CCTCGTCTGTCTCCACTTCC
pufN3	GCCCAAAGACTTCTCTGCAA	GCGCGTGTATTAAATGCTTTG
pufC1	AACAAGAGCGAGCGGTTTAG	CTGAGGGTGCTCCTTTTCCT
pufC2	CAAATTCTGGGTGGGCATACA	TTCGCTCGTCCCTAAGGACATTG
pufC3	GTCTCTTGGTCCACGCTGAT	CCTCGAAACACATCTCAATGC
Myc1	AGCATCACCACCAACAACAA	GGACCATCGTCCACCATATC
Myc2	CAGTTCCAGTTCGCAGTCAA	AGATAAACGCTGCTGGAGGA
ago1	TGATTACGTGCCTGCAGTTC	GGTGTGACCAACCAAAGTGC
ago2	AAGACGGGCGACTTTATACG	CGACGGCACAAATGAGTTTA
cycE 1	CGACTCGCACATTATCCAAA	CGGGGAAGCTTGAATCCTA
cycE 2	GGCATGGCCAACTATTCCTA	AATCACCTGCCAATCCAGAC