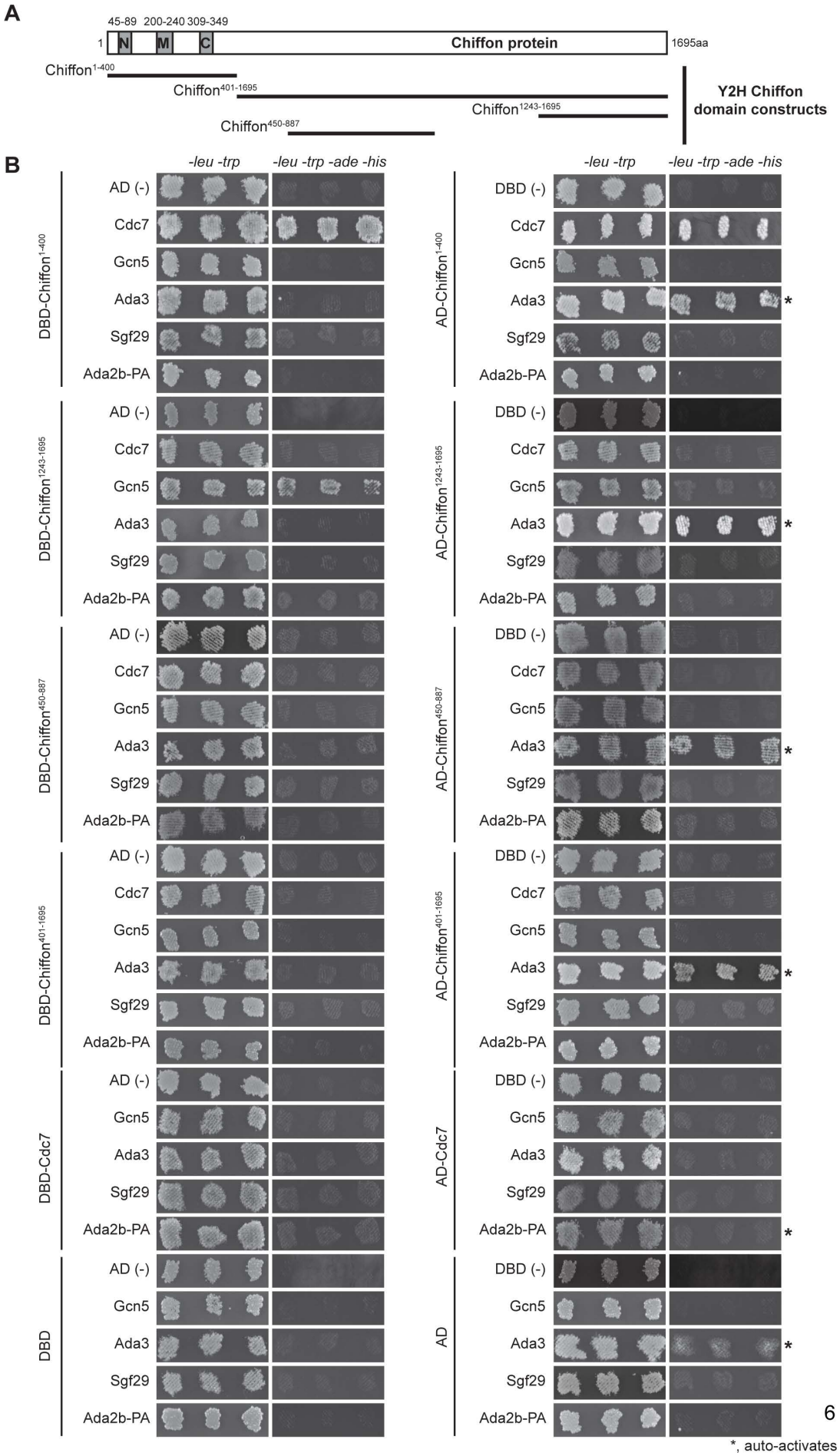

Figure S1. The N-terminal domain of Chiffon interacts with Cdc7, and the C-terminal domain of Chiffon interacts with Gcn5. (A) Schematic showing the Chiffon domains tested by yeast two-hybrid analysis. The conserved Dbf4 N, M, and C motifs are indicated by shaded grey boxes. (B) Yeast two-hybrid assay was performed to test the interaction of the CHAT subunits Gcn5, Ada2b-PA, Sgf29, Ada3, and Cdc7 with different Chiffon domains. The Gal4 activating domain (AD) or the Gal4 DNA binding domain (DBD) were fused to the indicated proteins. Empty plasmids expressing only the AD or DBD were used to test for auto-activation of each protein (*, auto-activation). Cells were patched on media lacking leucine and tryptophan to test for presence of the AD and DBD plasmids, and on media lacking leucine, tryptophan, adenine and histidine to test for interaction. Three independent transformed yeast colonies were patched for each interaction tested.

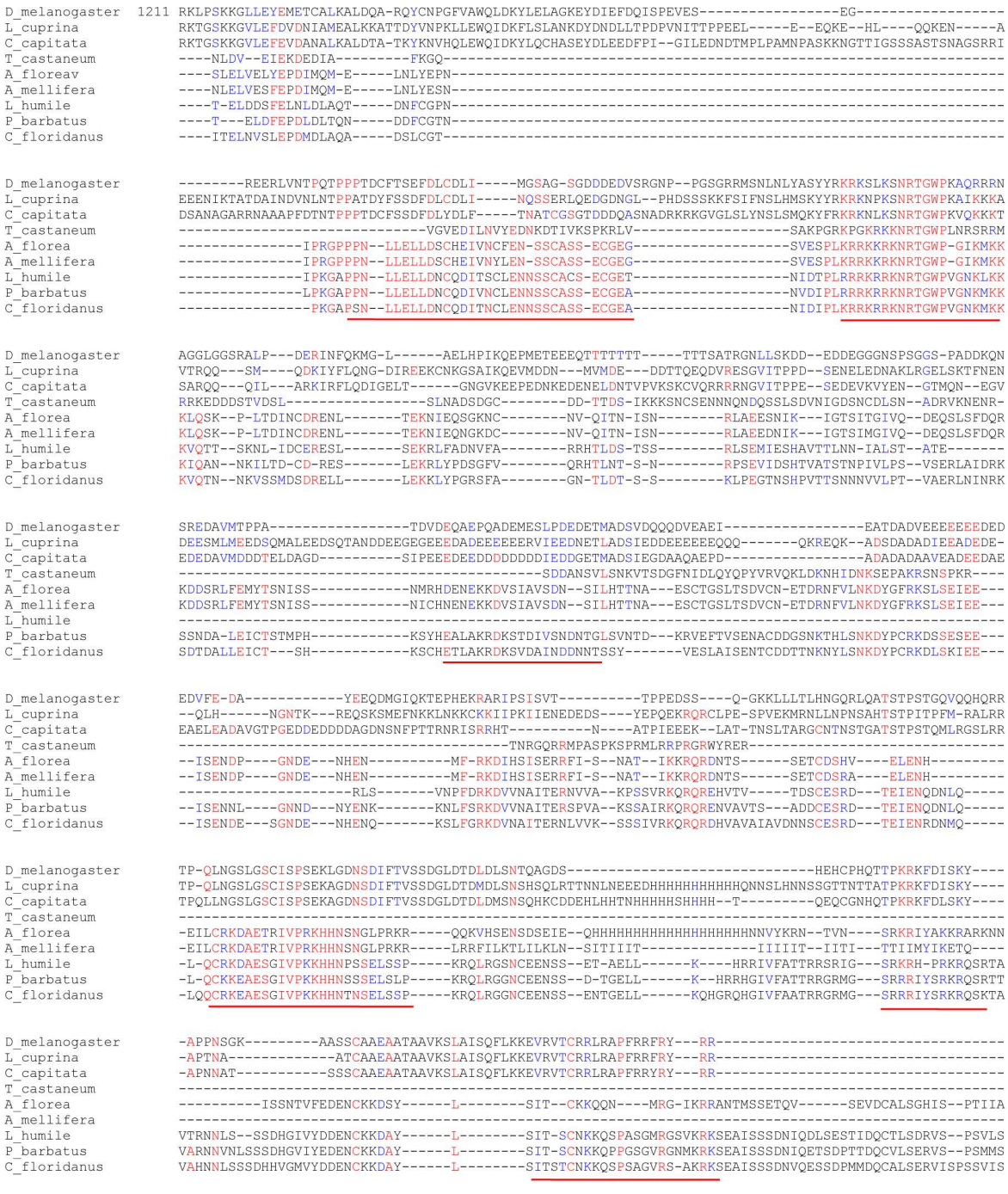

Figure S2. The C-terminal, Gcn5-binding domain of Chiffon shares regions of conservation within insect species. Insect Dbf4 homologs were aligned using Clustal Omega. The aligned region contains 1211 – 1695aa of *Drosophila* Chiffon, which includes the region that interacts with Gcn5 by yeast two-hybrid assay (1243 – 1695aa) and by co-immunoprecipitation using recombinant proteins (1400 – 1695aa). Regions of potential conservation within insects are underlined in red. Dbf4 homologs from the following insect species were used to generate this alignment: Diptera: *Drosophila melanogaster*, *Lucilia cuprina* (Australian sheep blowfly) and *Ceratitis capitata* (Mediterranean fruit fly). Coleoptera: *Tribolium castaneum* (Red flour beetle). Hymenoptera: *Apis mellifera* (Western honey bee), *Apis florea* (Dwarf honey bee), *Linepithema humile* (Argentine ant), *Pogonomyrmex barbatus* (Red harvester ant), *Camponotus floridanus* (Florida carpenter ant).

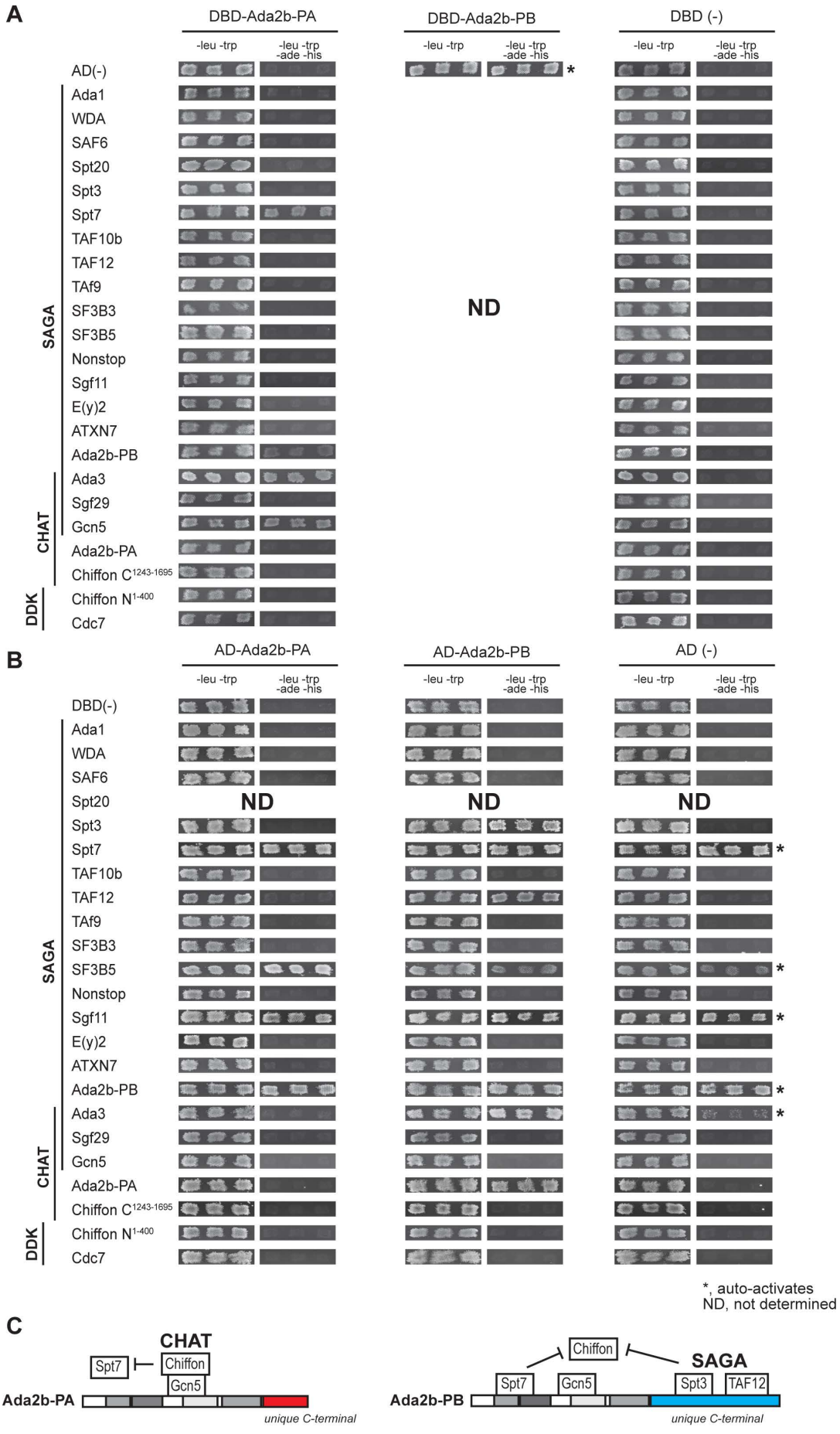

Figure S3. Ada2b isoforms interact with overlapping and distinct SAGA subunits. (A, B) Yeast two-hybrid assay was performed to test the interaction of Ada2b-PA or Ada2b-PB with the indicated SAGA subunits, Chiffon domains, and Cdc7. The Gal4 activating domain (AD) or the Gal4 DNA binding domain (DBD) were fused to the indicated proteins. Empty plasmids expressing only the AD or DBD were used to test for auto-activation of each protein (*, auto-activation). We did not test Ada2b-PB/DBD in combination with other SAGA subunits by yeast two-hybrid because Ada2b-PB auto-activated when fused to the DBD; in contrast, Ada2b-PA did not auto-activate. Cells were patched on media lacking leucine and tryptophan to test for presence of the AD and DBD plasmids, and on media lacking leucine, tryptophan, adenine and histidine to test for interaction. Three independent transformed yeast colonies were patched for each interaction tested. ND, not determined. (C) Model for differential binding of Ada2b isoforms with SAGA or CHAT. Both Ada2b isoforms bind Gcn5 and Ada3 and can interact with Spt7 by yeast two-hybrid; however, only Ada2b-PB associates with Spt7 *in vivo*. By yeast two-hybrid analysis, Ada2b-PB, but not Ada2b-PA, interacts with Spt3 and TAF12. We propose that Ada2b-PB binds Spt3 and TAF12 via its unique C-terminal domain (highlighted in blue), stabilizing association of Spt7 with its common N-terminal domain. Binding of Spt3, TAF12 and Spt7 to Ada2b-PB promotes formation of SAGA and prevents Gcn5 from binding Chiffon, potentially via steric clashes. In contrast, Ada2b-PA does not interact with Spt3 or TAF12 because it contains an alternative C-terminal domain that lacks the necessary binding regions (highlighted in red). Although Ada2b-PA is capable of binding Spt7 via its N-terminal region, this association is destabilized in the absence of Spt3 or TAF12. Instead, in the absence of Spt7 binding, Gcn5 interacts with the C-terminal of Chiffon and promotes CHAT complex formation. It is possible that Chiffon binding to Gcn5 might also prevent Ada2b-PA from binding Spt7. Notably, Ada2b-PB, but not Ada2b-PA, auto-activates expression of the reporter genes when fused to the DBD, suggesting that the unique C-terminal domain of Ada2b-PB may also interact with yeast SAGA subunits. Although by yeast two-hybrid Ada2b-PA and Ada2b-PB showed an interaction in one direction (panel B, AD-Ada2b-PB + DBD-Ada2b-PA), we never observed peptide spectra for Ada2b-PA isoforms in Ada2b-PB purifications (Table S1), suggesting that this interaction does not occur *in vivo*.

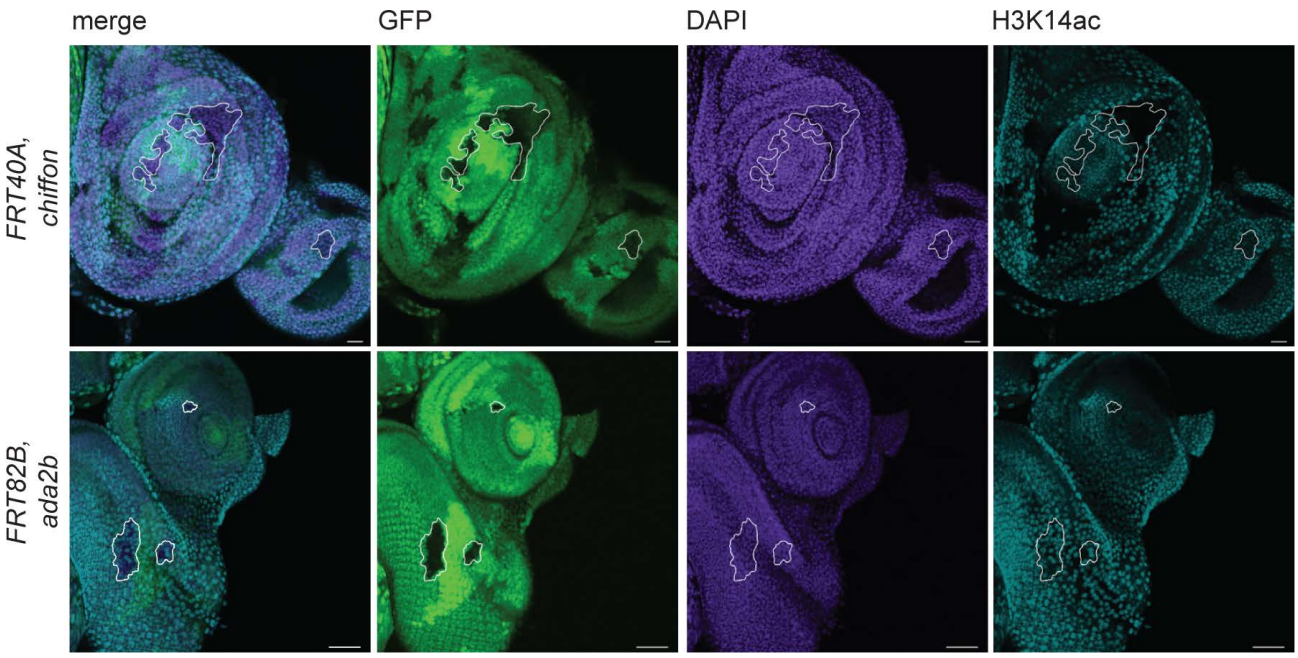

Figure S4. Chiffon is necessary for histone H3 acetylation in imaginal discs. Mosaic imaginal discs were generated using the FLP/FRT system for *chiffon*^{ETBE3} (n = 10) and *ada2b*^{1} (n = 3). Representative maximum intensity projection images for each allele showing α-H3K14ac and DAPI staining from imaginal discs containing multiple clones, marked by the absence of GFP and outlined in white. Scale bars, 20 μm.

Table S1: Distributed normalized spectral abundance factor (dNSAF) values for MudPIT analysis

Relative spectral abundance of SAGA, ATAC and CHAT subunits (rows) expressed as dNSAF (distributive normalized spectral abundance factor) in tandem FLAG-HA purifications from S2 cells using the indicated proteins as bait proteins (columns), relative to control purifications from untagged S2 cells (Control 1) or S2 cells expressing non-specific tagged protein CG6459 (Control 2). N or C epitope tagging indicated by -N or -C respectively.

											Bait protein:								
Subunit	**Complex**	**Isoform**	**FbgnID**	**CGID**	**Spt3-C**	**Spt20-C**	**Ada2b-PB-C**	**Ada2b-PB-N**	**Sgf29-C**	**Ada2b-PA-C**	**Ada2b-PA-N**	**Chiffon-C**	**Chiffon-N**	**Cdc7-N**	**Control 1**	**Control 2**	**Length (aa)**	**MW (Da)**	**pI**
Ada1	SAGA		FBgn0051866	CG31866	0.00333243	0.00734077	0.0220483	0.00466854	0.00352633	0	0	0	0	0.0000625	0	0	308	35399	7.5
WDA	SAGA		FBgn0039067	CG4448	0.00552565	0.01554854	0.01582185	0.00519146	0.00334123	0	0	0.00005353	0	0	0	0	743	83728	6.9
SAF6	SAGA		FBgn0031281	CG3883	0.00293458	0.00660909	0.01162017	0.0030241	0.00201973	0	0	0	0	0	0	0	717	79289	6.3
Spt20	SAGA		FBgn0036374	CG17689	0.00351628	0.02353081	0.00520999	0.00355825	0.00143588	0	0	0	0	0	0	0	1873	200987	9.5
Spt3	SAGA		FBgn0037981	CG3169	0.03087184	0.01201779	0.0090652	0.00291244	0.00235701	0	0	0	0	0.00002507	0	0	384	43506	9
Spt7	SAGA		FBgn0030874	CG6506	0.01000657	0.01604679	0.02432069	0.00870999	0.00590668	0	0	0	0	0	0	0	359	39460	5.4
TAF10b	SAGA		FBgn0026324	CG3069	0.00187468	0.00106069	0.02071581	0.00234493	0.00070849	0	0	0	0	0	0	0	146	15784	6.8
TAF12	SAGA		FBgn0011290	CG17358	0.00192448	0.00483938	0.00891659	0.00242505	0.00290922	0	0	0	0.00014176	0.00006016	0	0	160	17630	8.9
TAF9	SAGA		FBgn0000617	CG6474	0.00443045	0.00824435	0.01621665	0.00467974	0.00213947	0	0.00026061	0.00028613	0	0.00006925	0	0	278	29314	9.3
Nipped-A	SAGA		FBgn0053554	CG33554	0.00155267	0.01114666	0.00138525	0.00311948	0.00227211	0	0.00007646	0.00004198	0.00003591	0.00016762	0	0.00004722	3790	435332	7.8
SF3B3	SAGA		FBgn0035162	CG13900	0.00890875	0.01804809	0.03943939	0.00822185	0.00486846	0	0.00008857	0.00003241	0.00001848	0.00004707	0	0.00009724	1227	136616	5.5
SF3B5	SAGA		FBgn0040534	CG11985	0.00664134	0.01166006	0.02551194	0.00510183	0.00730157	0	0	0	0	0	0	0	85	9971	6.4
Nonstop	SAGA		FBgn0013717	CG4166	0.00220729	0.00924165	0.00023011	0.00340519	0.00135555	0	0	0	0	0	0	0	496	56441	8
Sgf11	SAGA		FBgn0036804	CG13379	0.00637129	0.02196486	0.00116462	0.00652114	0.0010555	0	0	0	0.00005786	0	0	0	196	21322	8.1
E(y)2	SAGA		FBgn0000618	CG15191	0.00423428	0.007973	0.00452009	0.00384166	0.00281641	0	0	0	0.00044913	0	0	0	101	11474	4.8
ATXN7	SAGA		FBgn0031420	CG9866	0.0008104	0.00344488	0.00229205	0.00126931	0.00151803	0	0	0	0	0	0	0	971	104206	7
Ada2b-PB	SAGA	PB (long)	FBgn0037555	CG9638	0.00280485	0.00971015	0.04832633	0.02245387	0.00550658	0	0	0	0	0.00001022	0	0	555	62031	7.1
Ada3	SAGA/CHAT/ATAC		FBgn0030891	CG7098	0.00181526	0.00562621	0.02863573	0.01100149	0.01181362	0.03476871	0.00762283	0.00901306	0.00006119	0	0	0	556	59916	7.4
Sgf29	SAGA/CHAT/ATAC		FBgn0050390	CG30390	0.0043802	0.01039546	0.02152324	0.01998087	0.15014752	0.06031126	0.01428933	0.01417476	0.00031392	0.00013323	0	0	289	32116	8.6
Gcn5	SAGA/CHAT/ATAC		FBgn0020388	CG4107	0.00340867	0.00963828	0.02386532	0.01426149	0.00483478	0.02637654	0.01265414	0.00772935	0.00018134	0.00002368	0	0	813	92169	8.9
Ada2b-PA	CHAT	PA (short)	FBgn0037555	CG9638	0	0	0	0	0.00196844	0.1763971	0.01351927	0.00846818	0.00001357	0.00004606	0	0	418	48316	8.8
Chiffon	CHAT		FBgn0000307	CG5813	0	0	0	0	0.00060455	0.00117305	0.0038109	0.02099009	0.02004294	0.00099012	0	0	1711	189217	8.5
Cdc7	DDK		FBgn0028360	CG32742	0	0	0	0	0	0	0.000207	0.00039772	0.00150666	0.04154105	0	0	700	78665	9.2
Atac1	ATAC		FBgn0031876	CG9200	0	0	0	0	0.00347668	0	0	0	0	0	0	0	356	40986	5.1
Atac2	ATAC		FBgn0032691	CG10414	0	0	0	0	0.00209881	0	0	0	0	0	0	0	774	89175	6
D12	ATAC		FBgn0027490	CG13400	0	0	0	0	0.00399154	0	0	0	0	0	0	0	969	111120	9.2

Table S2. Fly stocks used in this study.

Fly stock	Description	Genotype	Source/Reference
w^{1118}	Control untagged stock for embryo co-immunoprecipitations	*w*^{1118}	Bloomington BL3605
ada2b^{1}	Null *ada2b* allele removes both Ada2b splice isoforms	*ada2b*^{1}*/TM3, Sb*^{1} *Ser*^{1}	Mattias Mannervik (Qi et al., 2004)
ada2b^{842}	Null *ada2b* allele removes both Ada2b splice isoforms	*ada2b*^{842}*/TM3, Sb*^{1} *Ser*^{1}	Imre Boros (Pankotai et al., 2013)
ada2b^{1}*, FRT82B*	*ada2b, FRT82B* stock for somatic mosaic analysis	*ada2b*^{1}*, P{ry*$^{+t7.2}$*=neoFRT}82B/TM3 , Sb*^{1} *Ser*^{1}	(Stegeman et al., 2016)
Ada2b-PA	Transgene expressing Ada2b-PA isoform under *ada2b* promoter/enhancer	*yw; P{w*$^{+mC}$*=ADA2B-PA_ada2bEN}attP40*	This study.
Ada2b-PB	Transgene expressing Ada2b-PB isoform under *ada2b* promoter/enhancer	*yw; P{w*$^{+mC}$*=ADA2B-PB_ada2bEN}attP40*	(Weake et al., 2011)
chiffon^{ETBE3}	Null *chiffon* allele that deletes *chiffon* coding region: chr2L: 16344400 - 16351631	*chif*^{ETBE3}*/CyO*	John Tower (Landis and Tower, 1999)
chiffon^{ETBE3}*, FRT40A*	*chiffon, FRT40A* stock for somatic mosaic analysis	*chif*^{ETBE3}*, P{ry*$^{+t7.2}$*=neoFRT}40A/CyO*	(Stephenson et al., 2015)
Df(2L)RA5	Deficiency spans 35E1 – 36A1 including *chiffon* and *cactus* genes	*Df(2L)RA5/CyO*	Bloomington BL6915 (Landis and Tower, 1999)
chiffon^{DsRed}	CRISPR-Cas9 allele replaces *chiffon* coding region with 3xP3-DsRed marker: chr2L: 16344356 - 16349852	*chif*$^{DsRed\text{-}attP}$*/CyO*	This study
Chiffon-FL	Transgene expressing full-length Chiffon (1 – 1695aa) under *chiffon* 3' and 5' regulatory elements	*y w; P{w*$^{+mC}$*=Chiffon-FL*$^{1\text{-}1695}$*-NHACFLAG_chifp}attP2*	This study
Chiffon-C	Transgene expressing C-terminal domain Chiffon (401 – 1695aa) under *chiffon* 3' and 5' regulatory elements	*y w; P{w*$^{+mC}$*=Chiffon*$^{401\text{-}1695}$*-NHACFLAG_chifp}attP2*	This study
Chiffon-N	Transgene expressing N-terminal domain Chiffon (1 – 375aa) under *chiffon* 3' and 5' regulatory elements	*y w; P{w*$^{+mC}$*=Chiffon*$^{1\text{-}400}$*-NHACFLAG_ chifp}attP2*	This study
Chiffon-FL^{WF24}	Transgene expressing full-length Chiffon (1 – 1695aa) containing nonsense mutation at 174aa (c.520C>T; p.174Q>X) under *chiffon* 3' and 5' regulatory elements	*y w; P{w*$^{+mC}$*=Chiffon-FL*^{WF24}*-NHACFLAG_ chifp}attP2*	This study
*Chiffon-FL**	Transgene expressing full-length Chiffon (1 – 1695aa)	*y w; P{w*$^{+mC}$*=Chiffon-FL***-NHACFLAG_ chifp}attP2*	This study

	containing nonsense mutation at 376aa (c.1127C>A; p.376S>X) under *chiffon* 3' and 5' regulatory elements		
hs-FLP; FRT40A, ubi-nlsGFP	Heat-shock FLP, FRT40A GFP stock for somatic mosaic analysis	*y^1 w^{1118} P{ry$^{+t7.2}$=70FLP}3F;P{w^{+mC} =Ubi-GFP(S65T)nls}2L P{ry$^{+t7.2}$=neoFRT}40A*	This study
FRT40A; ey-FLP.N	FRT40A control stock for somatic mosaic analysis	*w^{1118}; P{ry$^{+t7.2}$=neoFRT}40A; P{ry$^{+t7.2}$=ey-FLP.N}6, ry^{506}*	Bloomington BL8212
ey-FLP;;FRT82B	FRT82B control stock for somatic mosaic analysis	*y^{d2} w^{1118} P{ry$^{+t7.2}$=ey-FLP.N}2 P{GMR-lacZ.C(38.1)}TPN1;;P{ry$^{+t7.2}$=neoFRT}82B*	Bloomington BL5619
hs-FLP;;FRT82B, ubi-nlsGFP	Heat-shock FLP, FRT28B GFP stock for somatic mosaic analysis	*y^1 w^{1118} P{ry$^{+t7.2}$=70FLP}3F;;P{ry$^{+t7.2}$=neoFRT}82B P{w^{+mC}=Ubi-GFP(S65T)nls}3R/TM6C, Sb*	Scott Hawley

Abbreviations: c., cDNA; p., protein

SUPPLEMENTAL REFERENCES FOR TABLE S2

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