#### Supplementary material

Article

#### Lysine Acetyltransferase p300/CBP Plays an Important Role in Reproduction, Embryogenesis and Longevity of the Pea Aphid *Acyrthosiphon pisum*

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**Figure S1.** Nymphs of *p300/CBP* dsRNA treated mothers. A prematurely born nymph (A) in ventral (A.1) and dorsal (A.2) view. The legs and antenna remain unfolded and the nymphs die after eclosion. In comparison, a viable nymph (B) in ventral (B.1) and dorsal (B.2) view.



**Figure S2.** The *A. pisum p300/CBP* mRNA sequence from clone LL01 contains the 5'-UTR, start codon and most of the open reading frame (ORF), but the stop codon and 3'-UTR were not represented in our assembly (Tab. S1). The RNAi target site is also shown. Additional RNAi target sides used in this study are indicated as non-overlapping fragments 1 and 2.



**Figure S3:** Life history parameters following the RNAi-mediated silencing of p300/CBP with the additionally tested non-overlapping fragments (called p300 non-overlap F1 and p300 non-overlap F2) as specified in Figure S2. We injected 40 individuals each with 25nl of a 3000 ng/µl solution. (A) survival, (B) total number of offspring, (C) average number of premature offspring, (D) number of viable offspring per day and (E) number of premature offspring per day is shown. Significances were tested using (A) the log-rank test, (B,C) the Kruskal-Wallis-test followed by Bonferroni corrections for pairwise analysis (\*p < 0.05, \*\*\*\*p < 0.0001) and (D,E) two-way-ANOVA (\*\*\*\*p< 0.000001).

 Table S1. Primer sequences used in this study.

	Primer name	Accession No. and residues (nt) sequences	Sequence	Frag. size (bp)	purpose
Eragment1	seq_AP_p300_fwd_1	MN734788	5'CTG ACC TTG GAC CGT GAG TT3'	807	sequencing
Fragmenti	seq_AP_p300_rev_1	nt1 – nt807	5'CAA ATT GAG AAA CAA AAT AGT TAA ACA3'	- 807	sequencing
Eragmont?	seq_AP_p300_fwd_2	MN734788	5'AAT CAG CCT CCG ACT TAG CA3'	956	soquencing
Fragmentz	seq_AP_p300_rev_2		5'CTG GTT GCG AAT TTG TTG TG3'	- 850	sequencing
Erogmont?	seq_AP_p300_fwd_3	MN734788	5'AGC AAC AAC AAA ACG GAC CT3'	007	coquencing
Fragments	seq_AP_p300_rev_3		5'TAC CTG GGG TTG TGA ATG TG3'	- 007	sequencing
Free and 4	seq_AP_p300_fwd_4	MN734788	5'CTC AAA TGC CAC ATC CTC CT3'	901	convensing
Fragment4	seq_AP_p300_rev_4		5'TTG GAA AAT GTA CCC TGT CTG A3'	- 801	sequencing
Energine ent E	seq_AP_p300_fwd_5	MN734788	5'TCA ATT CAA CCC AAT CAG CA3'	700	convensing
Fragments	seq_AP_p300_rev_5		5'CCA TTT TCT TTG AAC TTG AAC CA3'	- 769	sequencing
Erogmontf	seq_AP_p300_fwd_6	MN734788	5'AAC ACC GTC GGC ATC TGT GA3'	1002	sequencing
Fragmento	seq_AP_p300_rev_6		5'AGC AAC TTC ACC AGC GCC TG3'	- 1002	
Free cure e unt 7	seq_AP_p300_fwd_7	MN734788	5'ATT GGC TAT AAC AAA ATT AGG TAC G3'	020	convensing
Fragment/	seq_AP_p300_rev_7		5'TGC TCT TTC TCT GGC TGG AC3'	- 059	sequencing
Free and a mt 9	seq_AP_p300_fwd_8	MN734788	5'GAA GTT GAA ATT GGC CCT GA3'	965	sequencing
Fragmento	seq_AP_p300_rev_8		5'GCT GTG CTT GTT GCA GTC TT3'	- 805	
Erro ant O	seq_AP_p300_fwd_9	MN734788	5'TGT CCA GTG TTG TTC TGT CCA3'	011	sequencing
Fragments	seq_AP_p300_rev_9		5'GGA ACA TTT GCC TTT GTC GT3'	- 011	
Assembly	Fragment1-Fragment9	MN734788			Assembling
Reference	Protein	XP_008187184			
sequences	Nucleotide	XM_008188962			
	RNAi_AP_p300_fwd		5' <b><u>17</u> G TCT TGG TTA TTG CTG TGG TCG T3</b> '	267	DNA
	RNAi_AP_p300_rev		5' <b>T7</b> T CAC CGC GCT TCT TCA AAC AGT3'	- 507	NINAI
	RNAi_AP_p300_fwd_1		5' <u><b>T7</b></u> AGC CAC CTG TCC CAA GTC CA3'		RNAi non-overlapping
	RNAi_AP_p300_rev_1		5' <u><b>T7</b></u> GGT ACA CTT GTA GTT GAT GGC AC3'	_	Fragment 1
	RNAi_AP_p300_fwd_2		5' <u><b>T7</b></u> GCG CTG GTG AAG TTG CTA TAC G3'		RNAi non-overlapping
	RNAi_AP_p300_rev_2		5' <u><b>T7</b></u> GCA TGC CCA AAT GTG AGC CA3'	_	Fragment 2
	Rpl32_F		5'AGT ATC GCC CAA CAA TTA TCA3'		aDCB reference apre [1]
	Rpl32_R		5'CTT GAA TCG TCT TCG GAC T3'	-	deck reletence gene [1]
	qPCR_AP_p300_fwd4		5'GTT GCG AAC TCT AGG TCA GAA TA3'		aDCD torget ger -
	qPCR_AP_p300_rev4		5'GCT GTT GCA TTT GTT GTT GTT C3'	-	deck raiger gene

Table S2. Statistical analysis of RNAi data for survival compared to the GFP control.

Treatment	Mean survival [d]	Standard error [d]	Chi-square	Significance
Control (GFP)	13.88	0.44		
p300/CBP 3000 ng/µl	10.23	0.34	63.95	p<0.0000
p300/CBP 1000 ng/µl	10.26	0.39	49.03	p<0.0000
p300/CBP 250 ng/µl	10.93	0.40	38.21	p<0.0000
p300/CBP 50 ng/µl	11.08	0.47	30.65	p<0.0000

treatment	Mean start [d]	Standard error [d]	Chi-square	significances
Control (GFP)	4.93	0.07		
p300/CBP 3000 ng/µl	5.13	0.25	9	p=0.003
p300/CBP 1000 ng/µl	5.79	0.24	15.16	p<0.0000
p300/CBP 250 ng/µl	5.18	0.19	2.02	ns
p300/CBP 50 ng/μl	5.18	0.15	1.6	ns

Table S3. Statistical analysis of RNAi data for start of reproduction compared to the GFP control.

Table S4. Statistical analysis of RNAi data for average number of offspring compared to the GFP control.

Treatment	Mean number	Standard error	Kruskal-Wallis test value	Significances
Control (GFP)	62.38	1.84		
p300/CBP 3000 ng/µl	11.11	0.81	317.24	p<0.000
p300/CBP 1000 ng/µl	11.84	1.17	306.36	p<0.000
p300/CBP 250 ng/µl	21.38	1.42	209.96	p<0.000
p300/CBP 50 ng/μl	23.02	1.31	184.17	p<0.000

**Table S5.** Reproductive parameters evaluated during the RNAi experiments including viviparous offspring determined by two-way ANOVA.

Treatment		Statistical parameters	Significances
р300/CBP 3000 ng/µl	Aphid line (control vs p300/CBP)	df=1 mean square=463.702 F=98.235	p<0.0000
	Age of reproduction	df=28 mean square=349.784 F=74.102	p<0.0000
	Aphid line x Age of reproduction	df=27 mean square=262.781 F=55.67	p<0.0000
p300/CBP 1000 ng/μl	Aphid line (control vs p300/CBP)	df=1 mean square=2462.32 F=596.994	p<0.0000
	Age of reproduction	df=30 mean square=204.834 F=49.662	p<0.0000
	Aphid line x Age of reproduction	df=16 mean square=203.719 F=49.392	p<0.0000
р300/СВР 250 ng/µl	Aphid line (control vs p300/CBP)	df=1 mean square=1829.640 F=373.094	p<0.0000
	Age of reproduction	df=30 mean square=289.921 F=59.120	p<0.0000
	Aphid line x Age of reproduction	df= 17mean square=167.724 F=34.202	p<0.0000
p300/CBP 50 ng/µl	Aphid line (control vs p300/CBP)	df=1 mean square=1173.761 F=242.996	p<0.0000
	Age of reproduction	df=30 mean square=297.476 F=61.584	p<0.0000
	Aphid line x Age of reproduction	df=20 mean square=132.424 F=27.415	p<0.0000

Treatment	Mean number	Standard error	Kruskal-Wallis test value	Significances
Control (GFP)	0.43	0.05		
p300/CBP 3000 ng/μl	2.06	0.14	-185.09	p<0.000
p300/CBP 1000 ng/µl	2.98	0.17	-242.40	p<0.000
p300/CBP 250 ng/µl	1.89	0.24	-185.41	p<0.000
p300/CBP 50 ng/µl	1.57	0.24	-145.93	p<0.000

Table S6. Statistical analysis of RNAi data for average number of premature offspring compared to the GFP control.

**Table S7.** Reproductive parameters evaluated during the RNAi experiments including premature offspring determined by two-way ANOVA.

Treatment		Statistical parameters	Significances
p300/CBP 3000 ng/μl	Aphid line (control vs p300/CBP)	df=1 mean square=0.836 F=5.994	P=0.014
	Age of reproduction	df=28 mean square=2.472 F=17.722	p<0.0000
	Aphid line x Age of reproduction	df=21 mean square=3.745 F=26.848	p<0.0000
p300/CBP 1000 ng/µl	Aphid line (control vs p300/CBP)	df=1 mean square=8.954 F=41.950	p<0.0000
	Age of reproduction	df=30 mean square=1.702 F=7.973	p<0.0000
	Aphid line x Age of reproduction	df=16 mean square=3.119F=14.614	p<0.0000
p300/CBP 250 ng/μl	Aphid line (control vs p300/CBP)	df=1 mean square=3.658 F=27.031	p<0.0000
	Age of reproduction	df=30 mean square=0.823 F=6.083	p<0.0000
	Aphid line x Age of reproduction	df=17 mean square=1.237 F=9.144	p<0.0000
р300/CBP 50 ng/µl	Aphid line (control vs p300/CBP)	df=1 mean square=0.842 F=6.774	p<0.009
	Age of reproduction	df=30 mean square=0.643 F=5.171	p<0.0000
	Aphid line x Age of reproduction	df=20 mean square=0.924 F=7.435	p<0.0000

Table S8. Statistical analysis of RNAi data for body weight [mg] compared to the GFP control (dsRNA cor	centration
= 3000 ng/µl).	

Time after injection [d]	Body weigh	nt p300/CBP [mg]	Body weight GFP control [mg] Significan		
	Mean	Standard error	Mean	Standard error	Significances
0	1	0.02	0.92	0.02	ns
3	2.10	0.07	2.07	0.09	ns
8	2.86	0.09	2.89	0.07	ns

ns = not significant

**Table S9.** Statistical analysis of RNAi data for body size (length\*width)[mm<sup>2</sup>] compared to the GFP control (dsRNA concentration =  $3000 \text{ ng/}\mu$ ].

Time after injection [d]	Body size p300/CBP [mm <sup>2</sup> ]		Body size GFP control [mm <sup>2</sup> ]		Significancos
	mean	Std. error	mean	Std. error	Significances
3	2.43	0.08	2.45	0.1	ns
8	3.07	0.07	3.07	0.06	ns

ns = not significant

**Table S10.** Statistical analysis of RNAi data for body color (grayscale) compared to the GFP control (dsRNA concentration = 3000 ng/µl).

Time after injection [d]	Body color p3	olor p300/CBP - grayscale Body color GFP control - grayscale			Significances	
	Mean	Standard error	Mean	Standard error	Significances	
3	107.22	1.33	101.15	1.56	ns	
8	75.59	2.4	95.41	0.95	p<0.01	

ns = not significant

### **Fragment 1** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

# **Fragment 2** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

#### **Fragment 3** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

# **Fragment 4** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

#### **Fragment 5** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

# **Fragment 6** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

### **Fragment 7** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

### **Fragment 8** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

### **Fragment 9** of *A. pisum* p300/CBP mRNA, with primer positions underlined and indicated in bold (see Table S1).

#### Assembled fragments of A. pisum p300/CBP mRNA. Accession number: MN734788

TAAAACCATCCGTCGGACGAATTTCAGTAATTCCGGACGTTTGACGCGTTTGACCGGCCGTTTTGCCGATAAACGGTGACAGCACAGCGCGAACTATCGATAGTTGACGCGCTCCAAACAATCGTTGACCCGACTGCCGATGG CAGCCTCCGACTTAGCATAACATGGCCCATATTACTGTATGGTGATATCACATACTGCTTAATTATGTGACTTAACTATGTTTAACTATTTGTTTCTCAATTTTGAGATTTTAAGAATTTGGACATTGTAAAATAGGTTTAAGG CATTGTGCAATTTTTAATATGTGATTATTGATACTAGTAACTTTTTACTAGGTCTTTTCTGTTTTTAAATTCTTAAACTAAATATGTCAGACCTATTGGTGGATAGCCCTCCGAACAAGAGGCCAAAGTTGGAAGATCCATTT CAAGGGTCTTCTGACTCATCTGGTGTCTATTCTAGCAATGAGATGTTTGATCTCGAGAATGATCTCCCTGATGAGCTTATTAGCACTCACGTCTTGGAACCAGCAACCTGATATCAAGCCAAATCTTGGTCCTCAATTACCA ACCAGACTCCTCATCAACTTCATAGTTTGCCAATTAATTCTCATAGGTTACAAGGTTCCAATGGGTGCAATGAGTCCAGTGAATAATTTCAATGCATACAACAAATAATCAACAAATTAATGTTGTCCAACAACAACAACCAA TTGGTATTGTACGACAACCAGTACCGAGATTTAATGCCAACACTAATGGAATGTTAGTAGATACTCCGGTTCCTCCAGTCTCAAGCAAATGTACGTCTTGGACCACAGCAAGTTAATATGGTCAAATGGAAATGTAGTT GTACCACCTCAAGGAGTTCCCTCTGACCGTAGTCAAGCAAAATCCAGAAAAACGAAAAATGATAACCCAACAGCTTGTGCTATTATTACATGCACAATGTCAGAGACGAGATAATCAAGCTAATGGCGAAGTGAGAC AATGTCGTTTGCAACATTGTAATACAATGAAAGGAGTCTTAGCTCATATGACCAATTGCTTAGCTGGTCGAAATTGTAATGTAGCTCACTGTCGTCATCTAGACAAATAATATCACATTGGAAACAATGTACTAGACCAG CCTAACAATGTTGGTATACGAACGGCTTTGCAAATCTCCAAATGCCACATCCTCCTAATAACAATGTGCTGACACCTAATCAAAATACACGCGTATTAGTCCCACATTCACAACCCCAGGTAAAAAATACACGGGTATTAGTCCCACATTCACAACCCCAGGTAACAACTACAACTACAATGAGTGCAGAT CCCAGTACAAGTTCTGTAAATGAAATGCTGCAGTCTGCATCCGAACACAACTGTTCCAGTTCCAGTTCCAGCATCAACAATCTGCGACAAATATTCCTAATTGACAAATGACAACTAGTACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAATGACAATGACAACTAGTACAATGACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAACTAGTACAATGACAATGACAATGACAATGACAATGACAATGACAATGACAACTAGTAGTACAATGACAACTAGTACAATGACAATGACAACTAGTACAATGACAACTAGTACAAC TGAACAATCAAGCACAACCGAGTCAATCAATAACCAGGAAATCACATTGAAAGTAAGGAATGGCATGAGTCTATTACACCTGAACTAAGAAATCATTTAGTGCATAAAATTAGTTCAAGCAATTTTCCCAACATATGATCCT AAAGCAATGCTTGATAAACGTATGAATAATTTAGTGGCTTATGCTAGAAAAGTAGAAAGGTGATATGTACAATGTTGCGAACTCTAGGTCAGAATATTATCATAGGTTAGCCCAAACATTTTATAAAATACAAAAAGAGT GGAAGAAAAAATGCAAAAGCGAAAAGGAACAACAACAACAAAGCAACTGAGCAACTGGGAACTCAGCAACCGACTAATAATATCAATGCACAACTGCGTCTCCCAACTGCGCTCTACACAAGGATTGCCTACTCAGCAGCAACCG ATCACAACCACAGCAGCAGCAACAACCTTTACATCATACTTCAATTCAACCCCAATCAGCAAAATTTGCCACAACAAGTAGTAACAACAACATCTGGTCTAATGAAAACACAAAATGTCAGACAAGGGTACATTTTCCAATCACTTC AATCAAATGTTACAACCAAATAGACTTAATCAAATGAATAATGGAAAAAATGGTTAATGGACCAAGTTTGATAGCGAATAATACTGTGCCATCAACTAACAAGTGTACCATCAATGATGCCCAATAGTATGGGTAAAGGCT TGCTTATGGAAAAGGTAAAATGAATTCTGAAACACCGTCGGCATCTGTGAAATCTGAAATCTGAAACAAGAACGCGACGATAATGGTTCAAAGAAAATGGAAGTTAAAAATTAAAGATGAACCTATGAGTCCTAA CTGAATCAGATCCTTTTAAACAGCCTGTTGATCCTCAAGCACTTAATATACCCGACTATTTCATAATCATCAAAAAAGCCAATGGATTTATCAACAATTCGAGAAAAATTGGATACTGGTCAATTTCTGACCCTTGGAGCTAA GCTGTAACATTGGGCGAAGACCCTACAAGAGCCCAACAAGTAATTAAAAAAGAACAATTTATGGAAAATGAAAAATGATCATCTAGAATTGGAACCATTCATATTATGTACTCATTGTGGAAGAAAAACTCCATCAAAATA AAGAGTGAACAATTTCCTTAAGAAAAAAGAAGAAGCAGGCGCTGGTGAAGTTGCTATACGTGTAGTGTCCAGTTCAGATAAAATAGTTGAAGTAAAACCTGGGATGCGTAGTCGCTTTGTTGATAATGGAGAGATGC CGAATTTCCTTACAGGGCAAAAGCACTTTATGCATTTGAAGAAATTGATGGAACTGATGTTTGCTTCTTTGGTATGCATGTCCAAGAATATGGATCTGAAGCTCCGTCACCCAATACAAGAAGAGTTTATATTGCATGTTTGCATATTGCATGTCCAAGAATAGGATCTGAAGCTCCGTCACCCAATACAAGAAGAGTTTATATTGCATGTTTGCATGTCCAAGAATAGGATCTGAAGCTCCGTCACCCAATACAAGAAGAGTTTATATTGCATGTTTGCATGTCCAAGAATAGGATCTGAAGCTCCGTCACCCAATACAAGAAGAGTTTATATTGCATGTTTGCATGTCCAAGAATAGGATCTGAAGCTCCGTCACCCAATACAAGAAGAGTTTATATTGCATGTTTGCATGTCCAAGAATAGGATCTGAAGCTCCGACGCCACCCAATACAAGAAGAGTTTATATTGCATGTTTGCATGTCCAAGAAATTGGATCTGAAGCTCCGACGCCACCCAATACAAGAAGAGTTTATATTGCATGTTTGCATGTCCAAGAATAGGATCTGAAGCTCCGACCCAATACAAGAAGAGTTTATATTGCATGTTGCATGTCCAAGAATAGGATCTGAAGCTCGGACCCGATACAAGAAGAGTTTATATTGCATATTGCATGTCCAAGAATAGGATCTGAAGCTCGGACCCCAATACAAGAAGAGTTTATATTGCATATTGCATGTCCAAGAATAGGATCTGAAGCTCGGACCCCAATACAAGAAGAGTTTATATTGCATATTGCATGTCCAAGAATAGGATCTGAAGACTCGACGCTCCGACACCAAGAAGATTGACAAGAAGAGCT AGATTCTGTCAACCTTTTTCAGGGCCCAAACAGTACAGAACGTATGTTTATCATGAAATATTATTGGGTTACCTTGATTATGTAAAACAATTAGGATATACTATGGCTCACATTTGGGCATGCCCTCCATCTGAAGGTGACGA ACGGTAGAGATGCATTGTTGACCATGGCTCGAGAAAAACACTATGAATTTCATCACTTAGGAGAGCAAAGTTTTCTTCAATGGCAATGTTGTATGAACTCCATAATCAAGGACAAGATAAATTTGCTTACACATGTAAT 

#### References

 Sapountzis, P.; Duport, G.; Balmand, S.; Gaget, K.; Jaubert-Possamai, S.; Febvay, G.; Charles, H.; Rahbé, Y.; Colella, S.; Calevro, F. New insight into the RNA interference response against cathepsin-L gene in the pea aphid, Acyrthosiphon pisum: Molting or gut phenotypes specifically induced by injection or feeding treatments. *Insect Biochemistry and Molecular Biology* 2014, *51*, 20–32.