

Histone deacetylase 3 is a crucial regulator for metamorphosis and muscle development in the red flour beetle, *Tribolium castaneum* (Herbst)

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Additional file 2 Excel file: Details of 741 DEG identified after HDAC3 knockdown (P-value <0.05, 2 fold)

Additional file 3 Excel file: Details of 148 DEG identified after HDAC3 knockdown (P-value <0.01, 4 fold)

Additional file 4 Excel file: A) Details of common upregulated genes identified from HDAC3 knockdown larvae and TSA treatments (TcA cells). B) Details of common genes identified among dsHDAC3 (upregulated) vs. dsCBP (downregulated) vs. TSA (upregulated)

Additional file 5: Complete images of Western blots shown in Figure 6

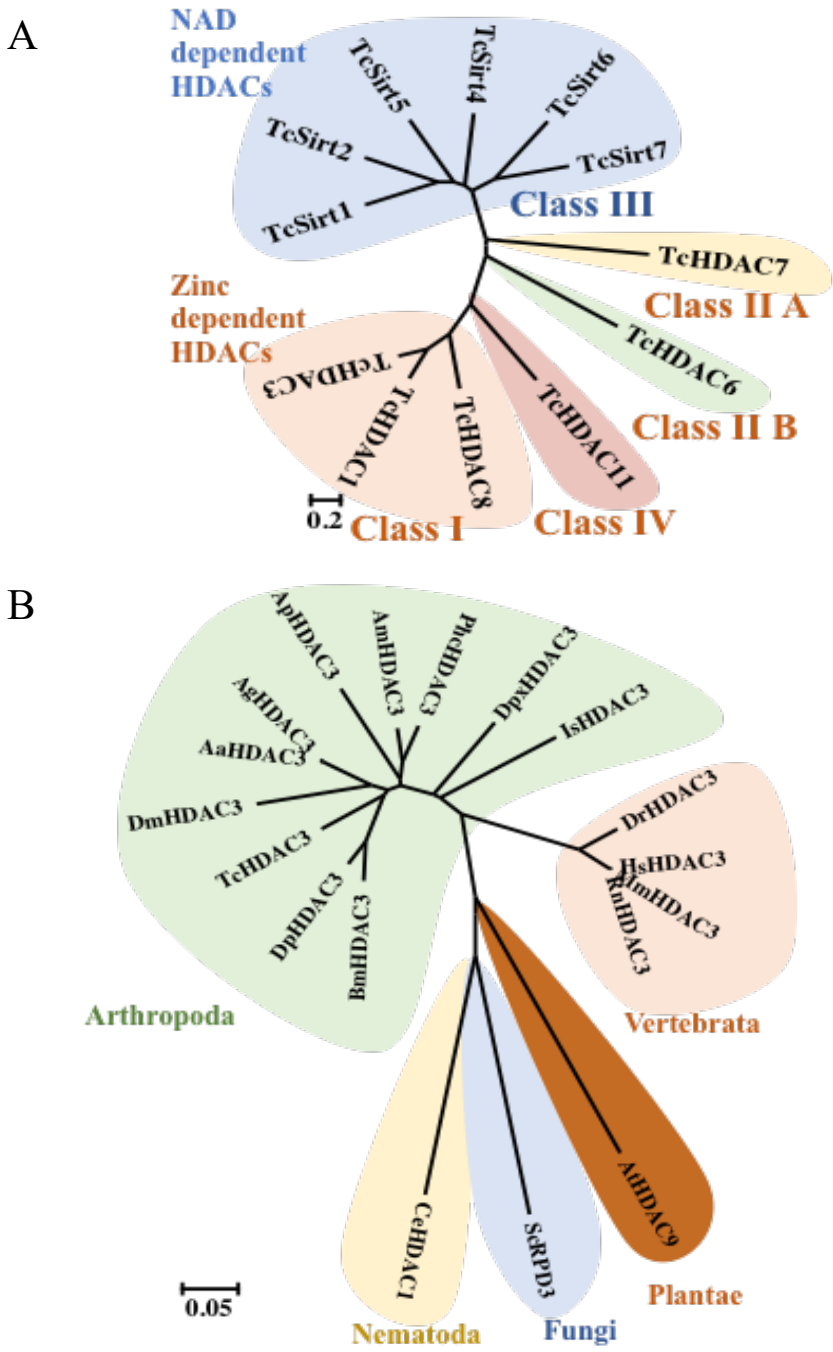


Fig. S1. HDAC3 is conserved and universal.

A) **Phylogenetic tree of HDACs families in the *T. castaneum*.** The evolutionary HDAC tree was inferred using the Neighbor-joining method. Evolutionary analyses were conducted in MEGA7 [1]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the

Poisson correction method and the units of the number of amino acid substitutions per site. B) Neighbor-joining phylogenetic tree of HDAC3 constructed using the amino acid sequence of proteins from selected organisms. (**Arthropoda:** *Drosophila melanogaster* (Dm) (Insecta: Diptera), *Ixodes scapularis* (Arachnida: Ixodida), *Pediculus humanus capitis* (Insecta: Phthiraptera), *Apis mellifera* (Insecta: Hymenoptera), *Tribolium castaneum* (Insecta: Coleoptera), *Acyrtosiphon pisum* (Insecta: Homoptera), *Bombyx mori* (Insecta: Lepidoptera), *Aedes aegypti* (Insecta: Diptera), *Anopheles gambiae* (Insecta: Diptera), *Daphnia pulex* (Crustacea: Water flea). **Vertebrata:** *Homo sapiens* (Hs), *Danio rerio* (Dr), *Rattus norvegicus* (Rn), *Mus musculus* (Mm). **Nematoda:** *Caenorhabditis elegans* (Ce). **Fungi:** *Saccharomyces cerevisiae* (Sc) (Yeast). **Plantae:** *Arabidopsis thaliana* (At).



dsHDAC3

dsma1E



Injected at last larval stage (0 h)



dsHDAC3- Injected after 3 days of last larval stage (72 h)

Fig. S2. HDAC3 knockdown induced pupal phenotypes.

Phenotypes observed in insects injected with dsHDAC3 into the last instar larvae. Developmental defects of wings with sclerotized areas were detected.

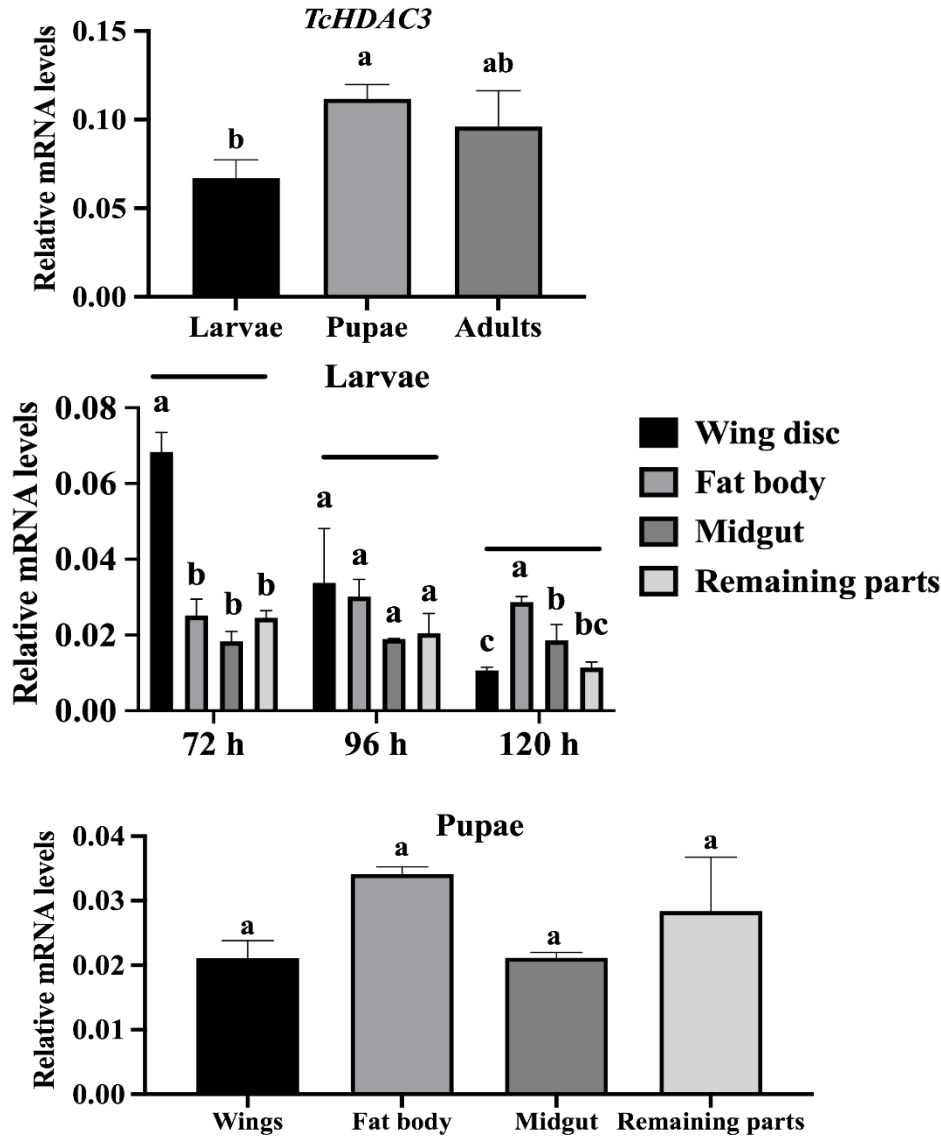


Fig. S3. Tissue-specific expression of HDAC3 in *T. castaneum* larvae and pupae. Samples were collected from 72 h, 96 h and 120 h (day 3- day 5) old last instar larvae, pupae and adults. RNA was extracted and converted cDNA and used to determine relative mRNA levels of HDAC3.

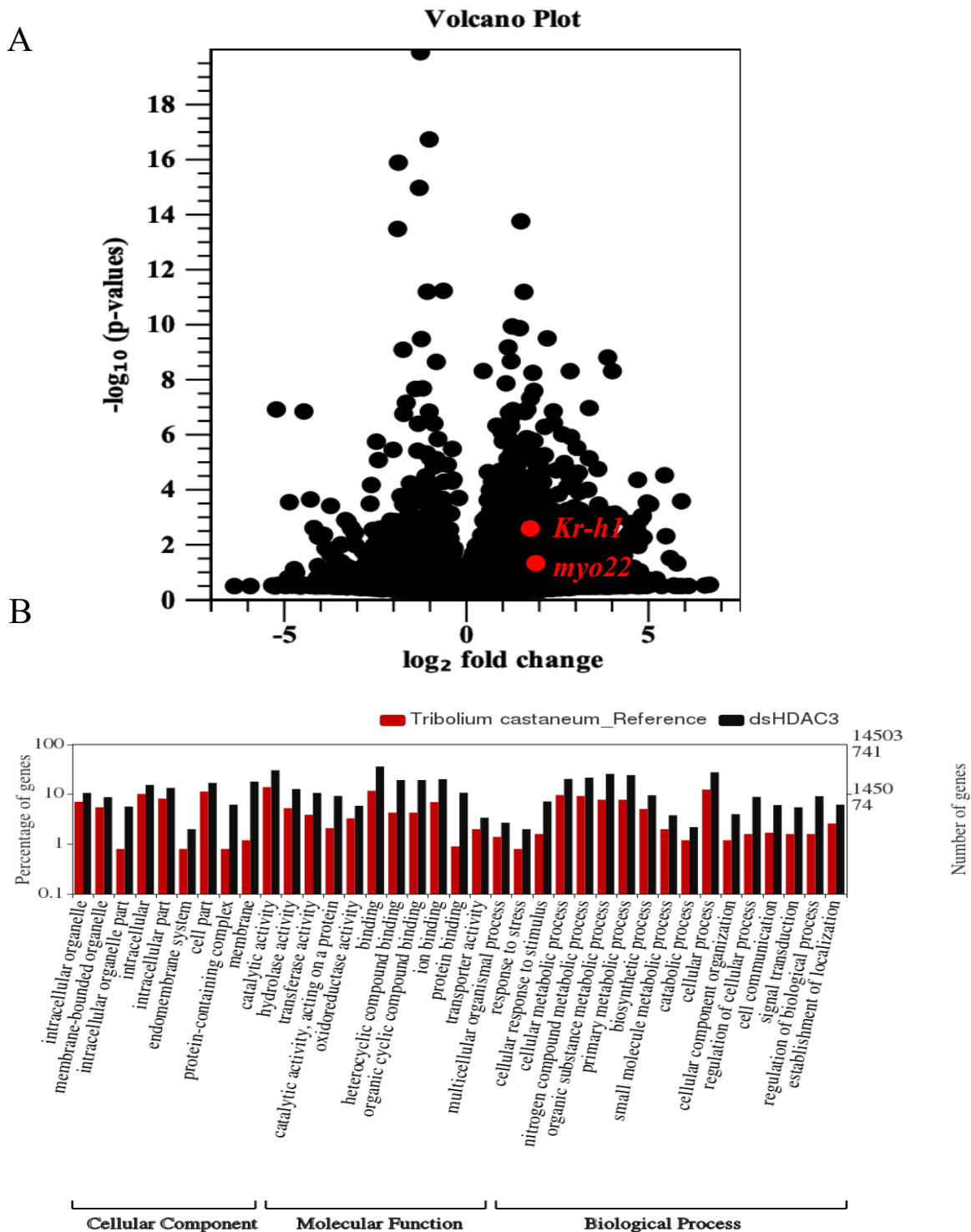


Figure S4. Volcano Plot and WEGO Plot. A) Volcano plot highlighting Kr-h1 and myo22. B) The WEGO analysis identified enrichment of various biological processes, molecular and cellular functions. The WEGO histogram shows the number and percent of genes in the cellular

component, molecular component, and biological function ontology classification group for *T. castaneum* transcriptome and 741 upregulated DEG genes in *HDAC3* knockdown larvae. The Blast2GO PRO-plugin software was used for the functional annotation of the transcripts.

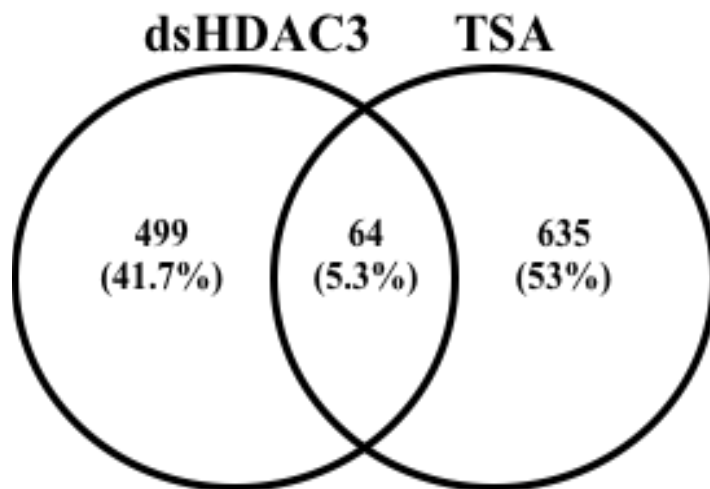


Fig. S5. Venn diagrams comparing the upregulated genes from dsHDAC3 (larvae) and Trichostatin A (TSA) treated TcA cells.

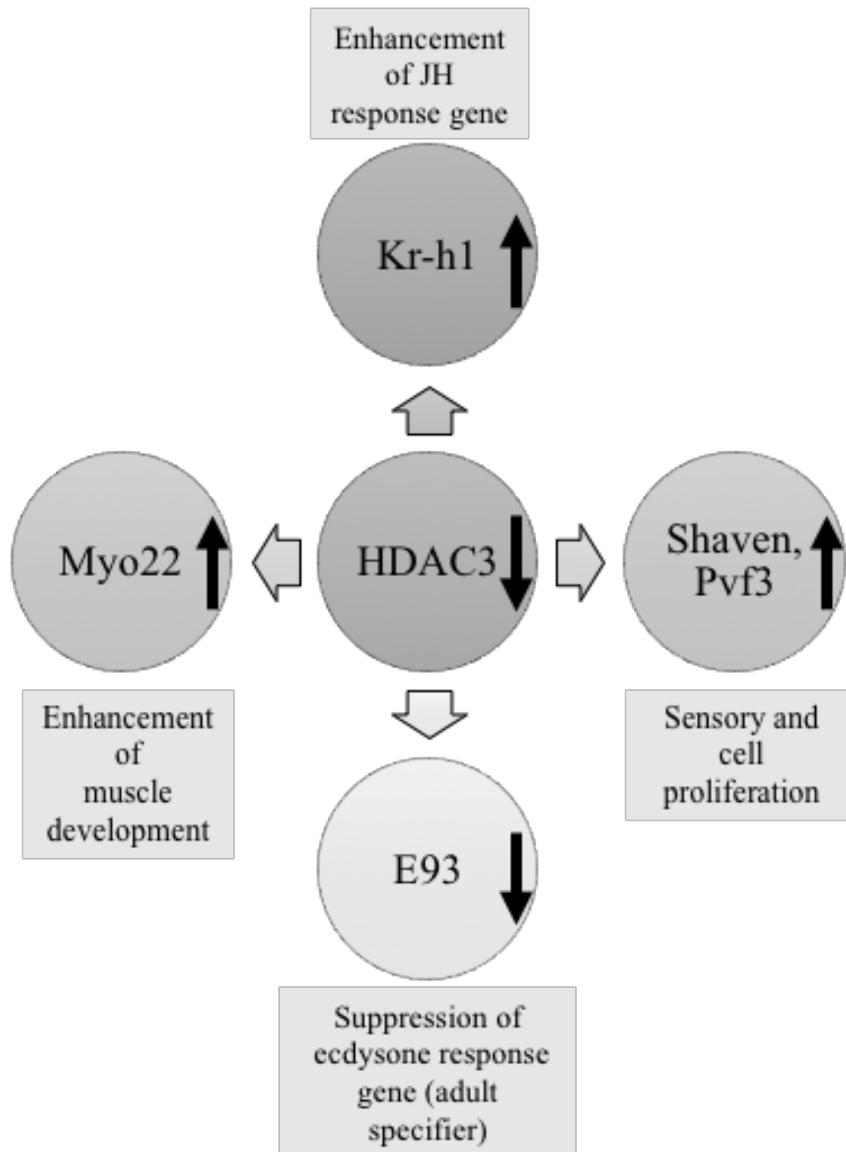


Fig. S6. Schematic representation of HDAC3 action in the red flour beetle.

JH response gene *Kr-h1* was significantly upregulated by HDAC3 knockdown; the ecdysone response gene *E93* was significantly down-regulated. *Myo22*, a myosin heavy chain gene essential for muscle development, is upregulated by HDAC3 knockdown. TC003570 (*Shaven*) and TC008417 (*pvf3*), which are essential for sensory and cell proliferation, significantly upregulated in pupae developed from HDAC3 knockdown larvae.

Additional Tables**Table S1: List of dsRNA and RT-qPCR primers used in this study.**

Gene	Primer
TcdsHDAC3F	TAATACGACTCACTATAGGGTGCAGATTTACCGGCCTTAC
TcdsHDAC3R	TAATACGACTCACTATAGGGATGACCCGATCAGTGAGATAGA
TcqHDAC3F	GAGACGCAATCCCTGAAG
TcqHDAC3R	TTTCGCCTCGACCATCTTA
TcqMyo22F	TTGCTCTCTCTCGCATGTTC
TcqMyo22R	GCCTTCTTCGGTCACGTAAT
TcqShavenF	GGAGTGTTTCGTTAACGGTAGG
TcqShavenR	CCCTCAGCTGCCTTGAAATA
TcqPvf3F	CCAGCTCCTGTCTCTTCTTTC
TcqPvf3R	CTCCAATGCGGCGTTTATTG
Tcq013029F	CTGATGAGCTTCTGGACGATAG
Tcq013029R	CTCGTCCAGTTCCAGCTAAAT
Tcq005670F	GAAGCCCTGCGACCAAATA
Tcq005670R	GTGTCGTCGTCGTTCTCTTTAC
Tcq012510F	CTCTCTGAAGCACCACAATGA
Tcq012510R	CGTTTCGACGTAGCCTTGAT
Tcq032605F	ACCGTACCAGTGTGAGAATTG
Tcq032605R	CGGAATAAGACCACACGTATCC
Tcq003005F	ATGCCTTCATCCAGCGTAAG
Tcq003005R	TTCACCGCTTGTAGAGGTTTC
Tcq000401F	AACGCCGACGTCACTTATTC
Tcq000401R	GCGCTCGTAGTCCAACATATC

Table S2: Summary statistics of RNA-sequencing data

A. Run Summary

Lane	PF* Yield (bp)	Number of PF* Clusters**	Q30%	Average Quality Score
7	15,963,128,520	313,002,520	95.64	39.04

* PF: Passed Filter. ** For single-read sequencing (SR), number of reads = number of clusters.

B. Read count statistics

Samples	Read Count	Single, mapped %	Reverse % of reads mapped
MalE-1	12,545,282	51.11	80.56
MalE-2	16,562,495	45.23	82.45
MalE-3	23,183,581	53.19	82.30
HDAC3-1	14,874,511	65.17	84.52
HDAC3-2	19,121,057	40.97	83.11
HDAC3-3	8,396,093	49.12	83.07

Table S3: Expression changes of hormone-related genes identified by RNA-seq analysis

Gene symbol	Gene description	fold change ^a	P-value ^b
<i>Kr-h1</i>	Krüppel homolog 1	3.36	0.00
LOC655046	Ecdysone induced protein 78C	16.06	5E-09
LOC660434	Broad-complex core protein isoform 6-like	2.40	0.03
LOC103313779	Nuclear receptor corepressor 1	2.69	0.00
LOC658947	Nuclear hormone receptor FTZ-F1 beta	2.04	0.00

^aExperiment - fold change

^bBaggerley's test: normalized values

Table S4. The list of select differentially expressed genes identified by RNA-seq and verified by RT-qPCR in *HDAC3* knockdown larvae.

Aliases	Fold change	Description	Domain
TC000110	5.2439	transcription termination factor 2	HELICc, DEXDc, SNF2_N
TC001775	2.8193	nuclear factor 1 C-type	MH1 superfamily, Nfl_DNAbd_pre-N
TC001921	6.4407	lethal (3) malignant brain tumor-like protein 4	MBT, SAM_Scm-like-3MBT3,4, zf-C2HC, SAM
TC003720	2.3232	pangolin	SOX-TCF_HMG-box, CTNNB1_binding superfamily
TC003972	3.9383	apterous a	Homeobox, LIM2_Lhx2_Lhx9, COG5576
TC004015	8.8793	uncharacterized LOC655848	MBF2
TC006021	2.6888	nuclear receptor corepressor 1	SANT superfamily
TC006040	2.3370	homeobox protein PKNOX2	Homeobox_KN, Meis_PKNOX_N
TC008129	2.3262	N-alpha-acetyltransferase 30	Acetyltransf_1, RimI
TC011468	2.9368	mortality factor 4-like protein 1	MRG, Tudor-knot
TC012866	4.6624	FACT complex subunit Ssrp1	HMG_box, PH2_SSRP1-like
TC014548	6.2153	uncharacterized LOC100141687	Zf-RING_2
TC014708	3.7195	uncharacterized LOC663601 (rel)	RHD_dimer, RHD-n super family
TC015048	3.0371	zinc finger protein 184	COG5048, zf-H2C2_2
TC015577	2.1008	pleiohomeotic	zf-C2H2, COG5048
TC015843	3.1657	uncharacterized LOC659528	JHBP
TC016270	2.9061	kelch-like ECH-associated protein 1	BACK, BTB, Kelch, PHA03098
TC031140	3.4616	insulin-like growth factor 2 mRNA-binding protein 1	KH-I
TC034026	2.3965	broad-complex core protein isoform 6-like	BTB
TC012990	3.3629	Krüppel homolog 1	Zf-H2C2_2, COG5048

Table S5: Upregulated JH-response genes in *HDAC3* knockdown larvae

Gene symbol	Gene description	JH III		dsHDAC3	
		fold change	<i>P</i> -value	fold change	<i>P</i> -value
LOC659434	fibroblast growth factor receptor homolog 1	96	0.030	4.1	0.004
LOC100141923	sodium-coupled monocarboxylate transporter 2-like	203	0.004	3.8	0.009
LOC103314154	DNA primase large subunit	3.8	0.020	3.4	0.011
<i>Kr-h1</i>	Krüppel homolog 1	29.9	0.000	3.4	0.003
LOC659929	lachesin	121	0.010	3.1	0.049
LOC660154	projectin	14	0.030	3.0	0.000

^aExperiment - Fold Change (normalized values)

^bBaggerley's test: HDAC3 vs malE normalized values

Table S6: Zn-Finger (COG5048) domains identified among upregulated genes in dsHDAC3 treated larvae

Gene Symbol	Locus tag	Gene description	fold	
			change	<i>P</i> -value
<i>Kr-h1</i>	TC012990	Krüppel homolog 1	3.36	0.00
<i>Pho</i>	TC015577	Pleiohomeotic	2.10	0.01
LOC662411	TC015048	zinc finger protein 184	3.04	0.03
LOC662791	TC002961	transcriptional repressor CTCF	2.26	0.00
LOC103313118		gastrula zinc finger protein XICGF57.1	2.89	0.01
LOC103313190	TC032605	zinc finger protein 2-like	13.87	0.00
LOC103313193	TC002118	Krüppel like factor 6	2.30	0.01

Table S7. List of genes selected from Appendix C (comparing TSA induced genes (TcA cells) with upregulated genes in *HDAC3* knockdown larvae) for RT-qPCR verification

Locus tag	Gene Description	TSA-up		dsHDAC3-up	
		Fold change	P value	Fold change	P value
TC012510	uncharacterized LOC103314255	85.4897	0.0087	27.0809	0.0013
TC032605	zinc finger protein 2-like	4.2700	0.0000	13.8733	0.0026
TC003005	muscle M-line assembly protein unc-89	3.1123	0.0000	10.3084	0.0000
TC003570	shaven	2.5056	0.0001	7.7380	0.0223
TC008923	myo22	6.7952	0.0000	3.7507	0.0464
TC013029	neprilysin-11	2.5863	0.0000	2.0641	0.0356
TC008417	Pvf3	3.5201	0.0000	3.7258	0.0144
TC000401	FAT	6.0517	0.0000	2.2999	0.0104
TC005670	epidermal growth factor-like protein 8	4.2720	0.0036	2.3405	0.0000

Table S8. Common genes identified among those upregulated after HDAC3 knockdown, induced by TSA and down-regulated after CBP knockdown

Locus tag	Gene Description	TSA-up		dsHDAC3-up		dsCBP-down	
		Fold change	<i>P</i> value	Fold change	<i>P</i> value	Fold change	<i>P</i> value
TC003570	paired box protein Pax-5/sv/shaven	2.51	0.00	7.74	0.02	-2.07	0.00
TC008417	PDGF- and VEGF-related factor 3/Pvf3	3.52	0.00	3.73	0.01	-2.47	0.00
TC003987	Titin	5.46	0.00	2.75	0.00	-2.20	0.00
TC005670	Epidermal growth factor-like protein 8	4.27	0.00	2.34	0.00	-3.35	0.01
TC000401	FAT; Cadherin	6.05	0.00	2.30	0.01	-2.10	0.00
TC013029	Neprilysin-11	2.59	0.00	2.06	0.04	-6.09	0.00

References

1. Kumar S, Stecher G, Tamura K: **MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets**. *Mol Biol Evol* 2016, **33**(7):1870-1874.