

Supplementary Information

Title: Epigenetic modification of histones play important role in juvenile hormone action

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Supplementary Figure legends

Table S1: List of primers.

Table S2: Detailed RNA seq statistics. Table showing a summary of read statistics after Illumina Hi-seq 4000 sequencing for individual biological replicates in different treatments.

Figure S1: Correlation of gene expression levels of selected genes (15) by comparing both RT-qPCR and RNA-seq data. Individual log fold changes obtained by RT-qPCR and RNA-seq for each gene in the sample group.

Figure S2: Relative expression of the epi-factor domain-containing genes in MD (*dsmalE* + DMSO) vs. MT (*dsmalE* + TSA), MD (*dsmalE* + DMSO) vs. CD (*dsCBP* + DMSO) and MD (*dsmalE* + DMSO) vs. CT (*dsCBP* + TSA) comparisons. Heatmaps illustrating the relative expression of genes with the epi-factor domain, in all three treatments (MT, CD, CT).

Figure S3: Pathway enrichment analysis. A) Pathways enriched after TSA induction. B) Pathway affected due to CBP RNAi. Here, the negative impact is equivalent to the enrichment.

Figure S4: Relative expression of FoxO transcription factor after different treatments in TcA cells and CBP RNAi in larvae. The data shown are the mean + S.E. (n=4). (Letters represent significance at 95% CI).

Supplementary Excel S1: Excel sheet-containing details of 32 genes induced by JH III (MD or *dsmalE* + DMSO vs. MJ or *dsmalE* + JHIII) and their relative expression in all other treatments compared to control. Genes behaving like Kr-h1 showed separately in a second excel sheet.

Supplementary Excel S2: Excel sheet containing details of 699 genes induced by TSA (MD or *dsmalE* + DMSO vs. MT or *dsmalE* + TSA) and their relative expression in other treatments of interest such as MD vs. CD (*dsCBP* + DMSO) and MD vs. CT (*dsCBP* + TSA). Genes with epifactor domains showed separately in a second excel sheet. Expression of Met and SRC in all three treatments showed in another excel sheet.

Supplementary Excel S3: Excel sheet containing details of 456 genes suppressed by CBP RNAi (MD vs. CD) and their relative expression in all other comparisons such as MD vs. CT, MD vs. MJ, MD vs. CJ and MD vs. MT.

Supplementary Excel S4: Excel sheet containing details of 181 genes responded after CBP RNAi and subsequent JH III treatment.

Supplementary Excel S5: Excel sheet containing details of 602 genes responded after CBP RNAi and subsequent TSA treatment.

Supplementary Excel S6: Excel sheet containing details of gene clusters obtained after Venn diagram analysis shown in main figure 6B.

Supplementary Table S1

Gene	Primer sequence(5'-3')	Direction
qRT-PCR primers		
TcasGA2_TC010839	CAGGGCCAGGAAATCAAG	Forward
	GCTGAAGGCGTTGTAGAC	Reverse
TcasGA2_TC000148	AACGAGCTCAACAATGGG	Forward
	CGCCGTATTTGTCCGAAA	Reverse
TcasGA2_TC012853	TCTGGGTCTATGACGGTAAG	Forward
	ATTCCTGGCCCATGTTTG	Reverse
GB16984	GGTGTTCAAGTTCGGGTTC	Forward
	TCGATGAGTGTCTAGGG	Reverse
TcasGA2_TC013193	ACTGGAACCCACGAAACG	Forward
	TAGCCGAAGTCAGGGAAA	Reverse
TcasGA2_TC007094	ATCGCGTGTGTGGAAATC	Forward
	TCGAAAGTGGTGCCAAAG	Reverse
TcasGA2_TC006721	TCACGCCTAAGCAGTTTG	Forward
	CGGGAACCTGGTGACTTATC	Reverse
TcasGA2_TC011641	GGACGGAGACAGACAAATC	Forward
	GTCGCTCGTCGAAATATCC	Reverse
TcasGA2_TC003157	CGGTCTTCACACAGTTAG	Forward
	GATAAACCCACTTCCTGGTC	Reverse

Note: Primers for the rest of the genes such as 4EBP, CBP, TcasGA2_TC013402, Kr-h1, Rp49, Nuclear receptor coactivator 1(SRC), Methoprene-tolerant, dsCBP will be found in our earlier publications mentioned below:

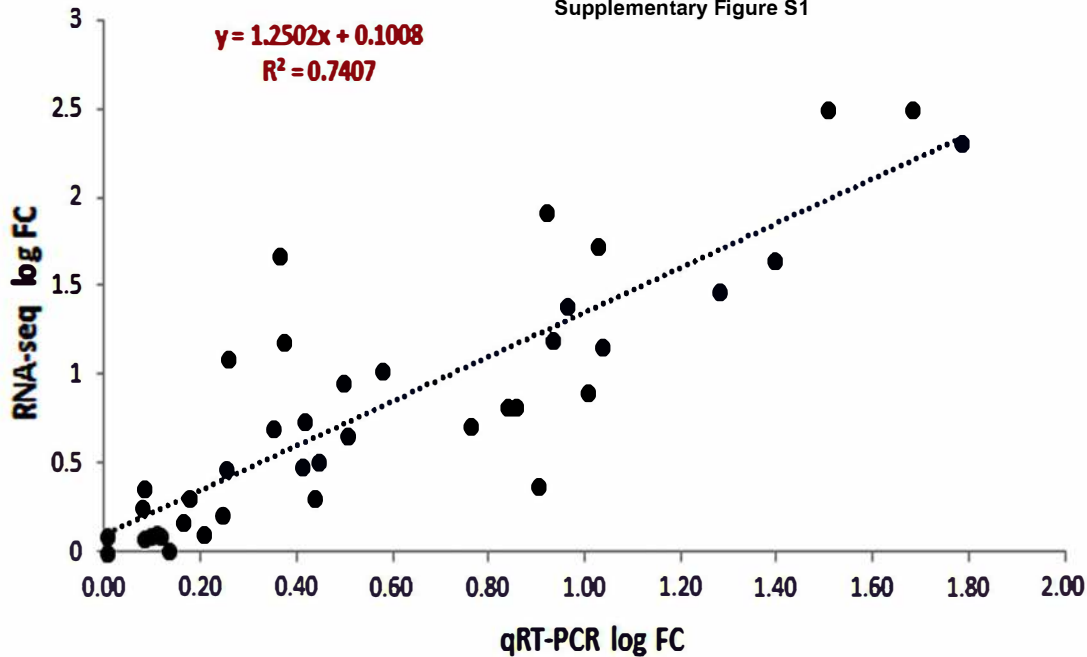
1. Roy A, George S, Palli SR: Multiple functions of CREB-binding protein during postembryonic development: identification of target genes. BMC Genomics 2017, 18(1):996.
2. Jingjing Xu, Amit Roy & Subba Reddy Palli: CREB-binding protein plays key roles in juvenile hormone action in the red flour beetle, *Tribolium Castaneum*. Scientific Reports 2018, 8:1426

Supplementary Table S2

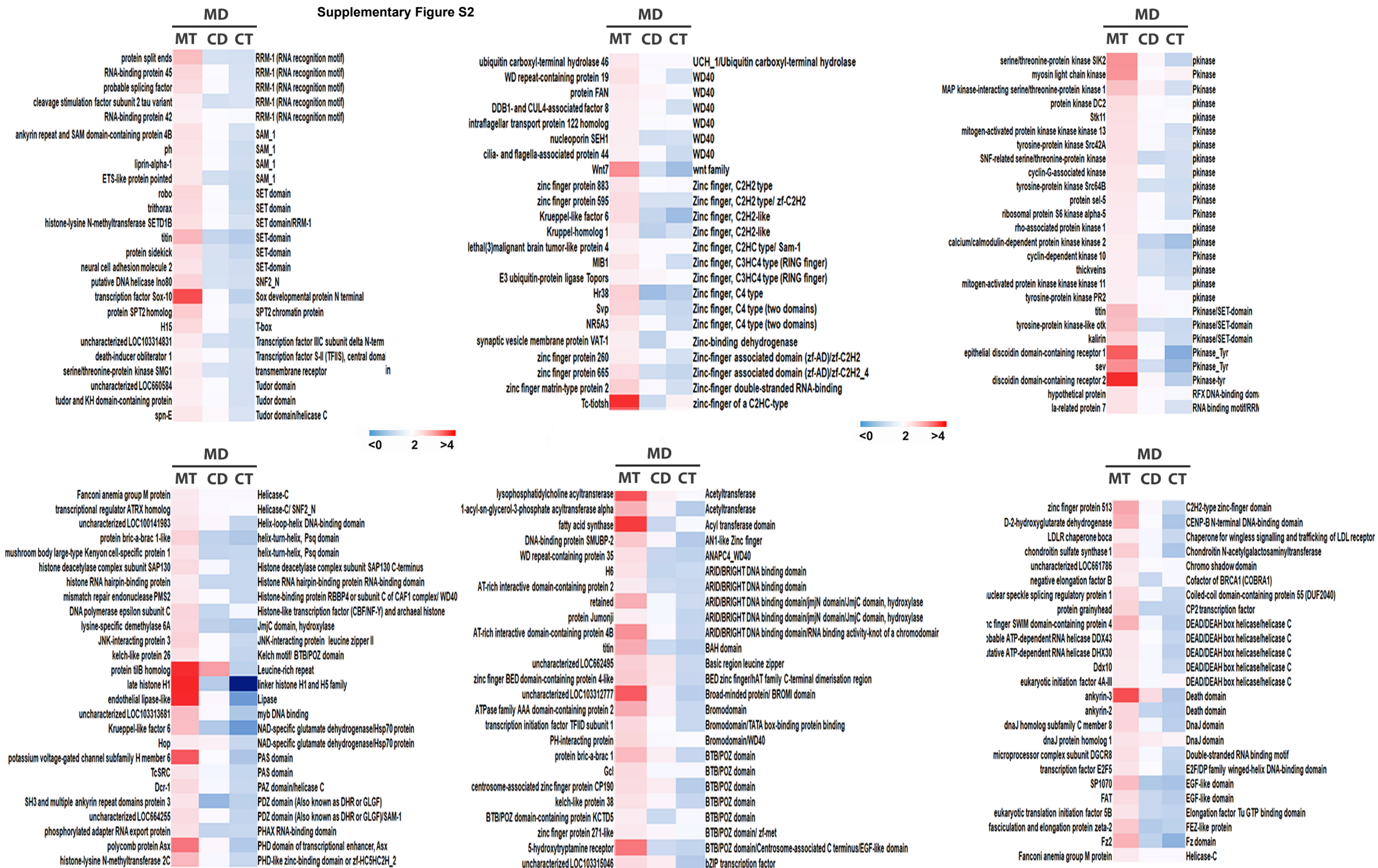
Sample	Total reads	Reads after QC*	% reads after QC	Mapped reads to Exon	% mapped	Uniquely mapped reads [#]
MD1	32,557,904	28,931,719	88.36	27,219,524	94.08	25,401,748
MD2	23,170,469	20,628,503	89.03	19,311,911	93.62	18,023,658
MD3	34,551,747	30,877,488	89.37	28,743,041	93.09	26,884,212
MJ1	20,396,843	18,501,186	90.71	17,210,535	93.02	16,035,311
MJ2	27,813,179	24,739,983	88.95	23,029,387	93.09	21,409,539
MJ3	15,842,254	14,225,539	89.79	13,338,160	93.76	12,450,612
MT1	33,863,136	23,336,656	68.91	21,159,900	90.60	19,738,084
MT2	25,746,558	21,219,162	82.42	19,009,018	89.58	17,686,189
MT3	26,406,181	10,372,230	39.28	8,661,657	83.51	8,123,452
CD1	32,579,815	29,284,406	89.89	27,312,718	93.27	25,754,030
CD2	32,777,122	28,905,897	88.19	27,790,577	92.68	25,257,369
CD3	33,049,791	27,857,657	84.29	26,227,606	94.15	24,742,314
CJ1	34,942,868	24,855,587	71.22	23,545,213	94.61	22,150,240
CJ2	26,406,828	21,384,097	80.98	20,146,622	94.21	18,937,081
CJ3	38,977,508	32,827,495	84.22	29,580,098	90.11	27,907,260
CT1	33,419,875	28,880,267	86.42	26,320,102	91.14	24,683,695
CT2	32,414,000	25,896,217	79.89	23,019,658	88.89	21,520,191
CT3	28,211,808	24,411,723	86.53	22,239,670	91.10	20,859,554

*QC= Quality control # only reads uniquely mapped to Exon regions are considered for gene expression analysis.

Supplementary Figure S1



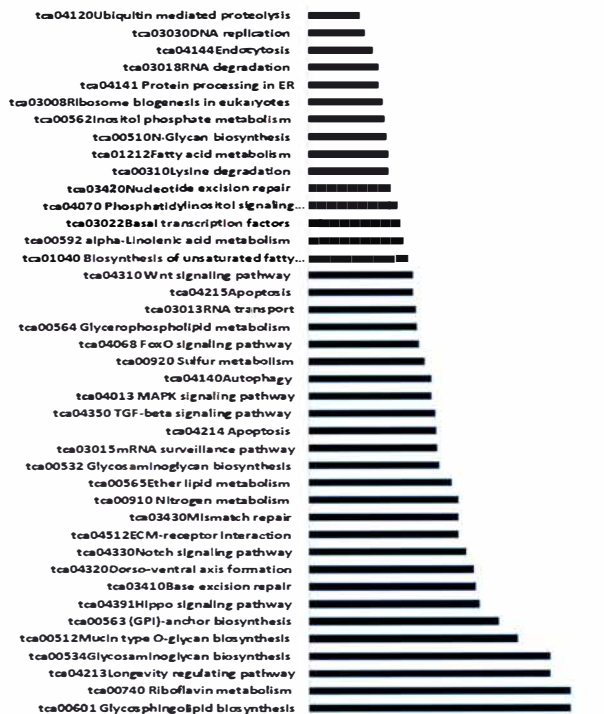
Supplementary Figure S2



A.

Impact of TSA induction

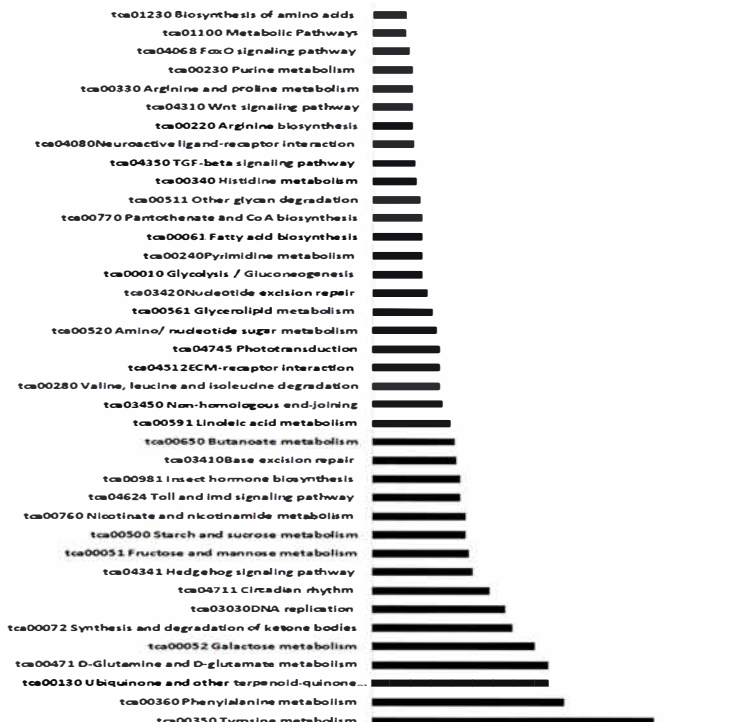
Pathways



MD Vs MT

B.

Impact of CBP RNAi



Times Enriched

MD Vs CD

Forkhead box protein O (TcasGA2_TC001062)

