Supplemental Information

to support:

Molecular Organization of *Drosophila* Neuroendocrine Cells by DIMMED
by D. Park *et al.*

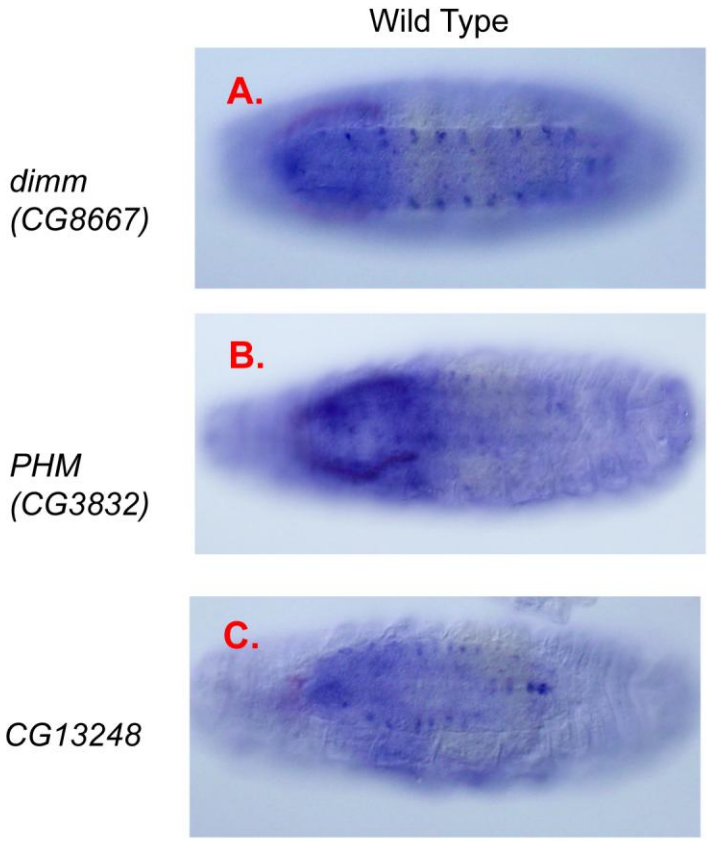

Figure S1. Similar spatial distribution of RNAs for *dimm*, *Phm* and *CG13248* in late stage control embryos. These data support the spatial analysis of DIMM target RNAs illustrated in Figure 2

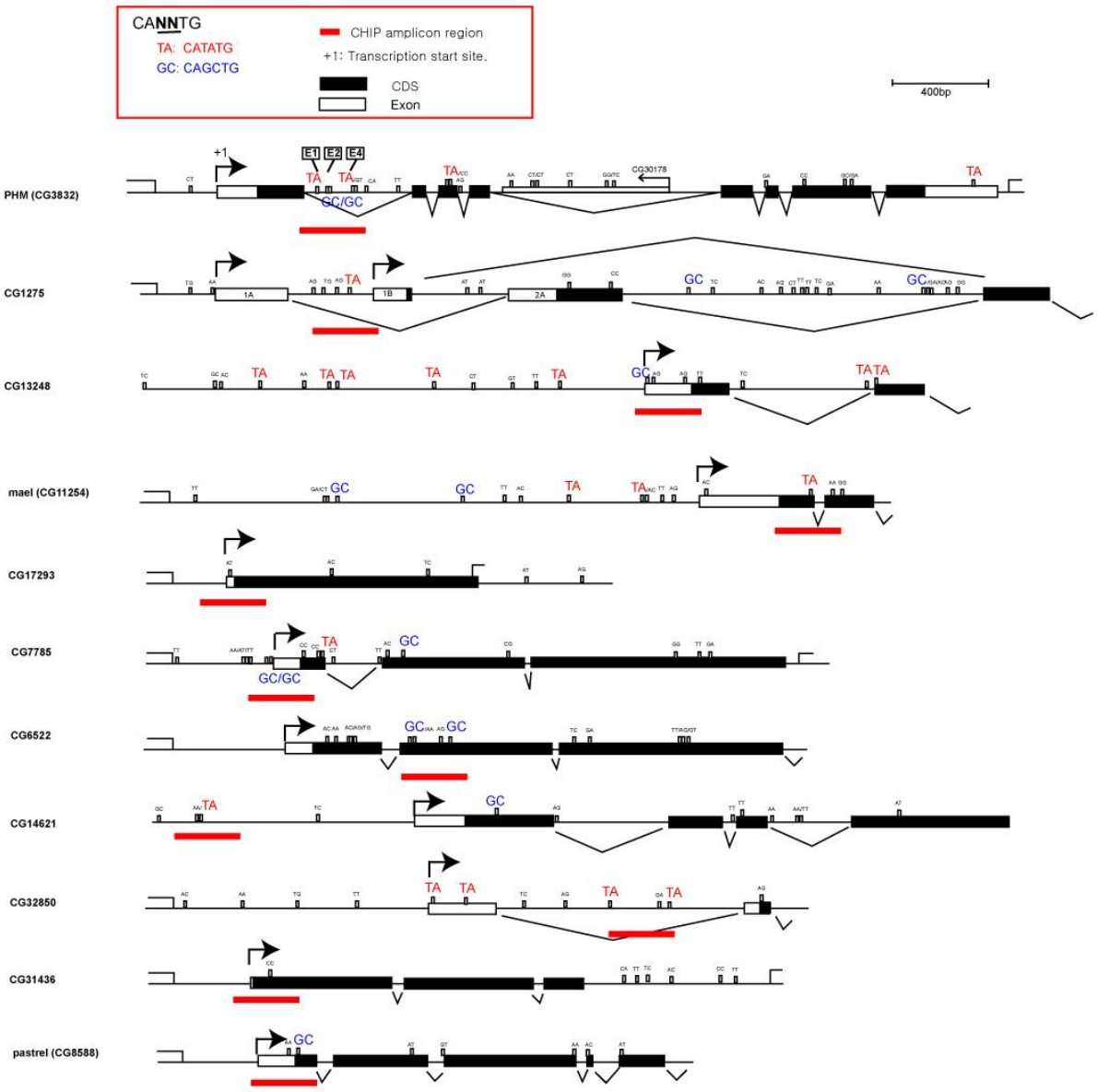

Figure S2. The distribution of CATATG and CAGCTG E-boxes within and around the candidate gene loci.

The schematic cartoon shows the putative DIMM binding sites within eleven candidate genes. Each box represents an E-box, CANNTG; Two nucleotides on the top of box indicate the sequence of NN. This information supports the study of DIMM trans-activation illustrated in Figure 3.

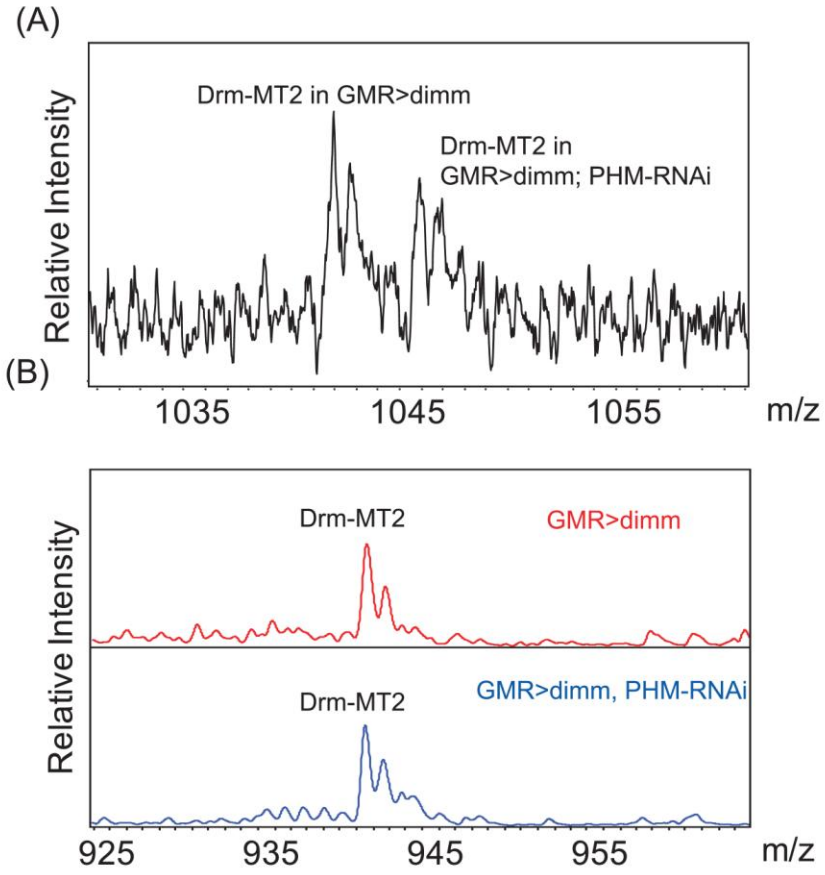

Figure S3. MS-based quantitation results from endogenous peptide Drm-MT2.

MALDI-TOF/TOF mass spectrum of labeled MT2 with light form of succinic anhydride in the control (***GMR*>UAS-*ppMII;* UAS-*dimm***) and with heavy form of succinic anhydride in the experimental (***GMR*>UAS-*ppMII*; UAS-*dimm*; UAS-*Phm-RNAi***) samples. The relative intensity ratio of MII in experimental versus control genotypes is 0.95. This data supports the analysis illustrated in Figure 5.

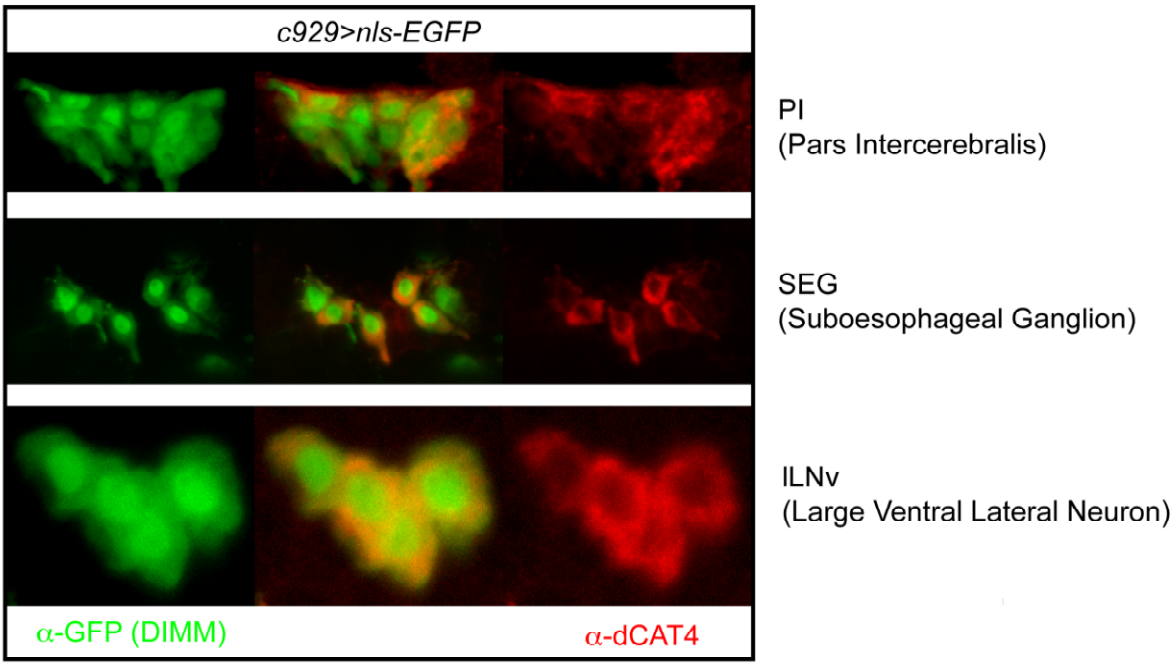

Figure S4. DIMM-positive adult brain neurons double stained for *c929*-GAL4 (green) and dCAT-4-like immunoreactivity (red). Note that the latter is primarily cytoplasmic and within individual cell bodies, appears heterogeneous (spotty). These images support the data presented in Figure 6.

Table S1. Information on primers used for analysis of cDNAs identified by genome-wide microarray screen of embryos following DIMM-over-expression.

GENE	Primers
Rp49-1	5'-GTTCGATCC GTAACCGAT GT-3'
Rp49-2	5'-TCGGATCGA TATGCTAAG CTG-3'
PHM-1	5'- GAGTCG AACTCC GCAACA GG
PHM-2	5'- TGATGG GCGTGC ACAAGT AC
CG11556-1	5'-TCGCATGACAGCTTTGAACG-3'
CG11556-2	5'AATCTGGCTGTGTCGCATCTG-3'
CG1275-1	5'-TTCAGAGTCTTCTTGCGCGTG-3'
CG1275-2	5'-CATCCGCTGTTCATGACCATT
CG1515-1	5'-TCCTTTTTCCAACGTGGCA
CG1515-2	5'-GGCATCCTGTTTCACCGATT
CG16918-1	5'-AGTTATGGCGAATCGAGGACG
CG16918-2	5'-ACGCTTTGGACGGAATGCTT
CG17293-1	5'-CCGTGACGGCAAAACAATTC
CG17293-2	5'-GGATAGCCCGTGAACGTTTGTA
CG33166-1	5'-GCCAGATCTCGTGACGCTTATC
CG33166-2	5'-TACTACGCCGATCGCTACACCT
CG5300-1	5'-AAGATGCCAGTGGACAAATCAC
CG5300-2	5'- TTTGTCGATGCAGTGGTGC
CG7224-1	5'- CAAAATGCAATCCGTGACCAG
CG7224-2	5'- CCTTGATCTCGACCACCATGTC
CG7779-1	5'- TGTTGGAGATCATGGAGCCAA
CG7779-2	5'- CCGGAGAACGATGTGGAGTATC
CG9994-1	5'- ACTACGCAGATGTGGACCAGGA
CG9994-2	5'-TTCGATGACGTCTCGATGTG G
CG13822-1	5'-GATGCTCTGCAGGACAACGACT
CG13822-2	5'-CGCCAGTGGATGTGTTGTTGTA
CG9893-1	5'-

	TTGCTCAACTCTGCACGCTTC
CG9893-2	5'-AAGCGATATGGCGAGATCCAG
CG11254-1	5'-CATGAAACGTACCATCGAGCG
CG112542	5'-ACATAGACGTCGGTGGTCAAGG
CG6522-1	5'-TAACGGCAACTTCGCCCAT
CG6522-2	5'-CCGGAACAGTTCCAACAGCTAC
CG18446-1	5'-ACAAGTATCTGGTTGCGCCAC
CG18446-2	5'-TGAATCGCAGTCCAGCCAT
	5'-
CG8588-1	TCCTTGGCAGCCTTGTCAAA
CG8588-2	5'-CACGCACAGCGTCTACAACTTT
CG1009-1	5'-AAACTCAACTTGGACTTGGCCA
CG1009-2	5'-TGGCGAGGAAGTGGAAACTCT
	5'-
CG11328-1	CCGTTCCCGGTATCATTGTCT
CG11328-2	5'-GCTCACCACCACACAGCAACTA
CG10679-1	5'-GCCAATACCAAGTGAAGAACGG
CG10679-2	5'-GAGAGTAGAGCCGAAACCCACA
CG5486-1	5'-GAGAGTAGAGCCGAAACCCACA
CG5486-2	5'-ACTAGAAGACCGCACCGTCAAC
CG8745-1	5'-TTCTT CGTCA ATTCC GGATC C-3'
CG8745-2	5'-ACCGA CTGCA GATGA CCATG A-3'
CG13248-1	5'- AAACC GATGC CTCAA CACCT T -3'
CG13248-2	5'- CCATT TCCTT GGCCA CAGTT C -3'
CG7785-1	5'- ACATA AGGAA ACCGA CGGCA-3'
CG7785-2	5'- TTACG CGCAG TTGGT GTGA-3'
CG14021-1	5'- TCCCT ACACC GTTAC CATGG A-3'
CG14021-2	5'- TGCCG ATCAC AAAGA CCGA-3'
CG14321-1	5'- GCGAC GTTTA ATTTT CCCGA-3'
CG14321-2	5'- TCGGA TGCAG TTGAT CTTGG-3'
CG18410-1	5'-AATGC CAAGA CAGAG AGCGA G-3'
CG18410-2	5'-TGATG GCCAA CCTCT GGTAG T-3'
CG33149-1	5'-CGGAG GCTAC AAATT CCACA A-3'
CG33149-2	5'-CCATG GCAGT TTTTG CAGAA-3'
CG5841-1	5'-GCGAT GCCAC TGAGG AATTT-3'
CG5841-2	5'-CATTG ACATC CGGTC CAGAA C-3'
CG30269-1	5'- GCACC CATCT GATAG CCTTG A-3'

CG30269-2	5'- TCGCA CATGC CACAG TAGTG A-3'
CG14621-1	5'-TTATC AGCGC CCTCA TCTCC A-3'
CG14621-2	5'-AAGCA AATGC AGCAG CCGT-3'

Table S2. Information on primers used for ChIP analysis.

Gene	Primer	Seq	bp	Purpose	Genome locus
CG7785	**85-E7**	GACCATCAGCTGGAAAAAGAG	21	E-BOX	>chr3R:13847304+13847367_64bp
	85-E8	ATTTTCGCTTCTTCGCTTGG	20		
CG7785	**85A5**	GCTTTCCTCTTGCCCAAAAA	20	NEG_CONTROL	>Chr3R:13841517-13841566_50bp
	85A6	CCGAATGATGATGCCTCTTAAAC	23		
mael	**msr9**	AGGCTGTTTCCCACTGCG	18	E-BOX	>chr3L:22717754-22717804_51bp
	msr10	AGCATCGACCAAAACTTACCTCC	23		
mael	**msr13**	AACGATCTGCGGCACCATAT	20	NEG_CONTROL	>3L:22712689-22712758_70bp
	msr14	GCCACTCGGTGTAGGCATTAA	21		
CG1275	**CY1**	GCCAAATGAAATGGCCAAGT	20	E-BOX	>chr3L:2256810-2256919_110bp
	CY2	AGCTGGGCCTTCCATATGG	19		
CG1275	**CY7**	TTGCACAACTGGTGACCAAA	20	NEGCONT_(3'_UTR)	>chr3L:2252875+2253033
	CY8	CAAACGAAACCGAACCTGAT	20	~3,800bp_away_3'	
CG6522	**PET1**	TCTCCAAGGCGCACAACA	18	E-BOX	>chr2R:13355910-13355980_69bp
	PET2	GCTGCAGCAATACGTTCAGAAA	22		
CG6522	**PET5**	GCACGACGGTGGGAAAGTAA	20	NEG_CONTROL	>chr2R:13349761-13349830_70bp
	PET6	CCGACTTCTTCTGATTGCAAAA	22		
pastrel	**PST3**	CAGTTGTGCTGCTAATGGAGTTG	23	E-BOX	>chr3L:7353152-7353222_71bp
	PST4	CGTTCAATTAGTGTGCGCCTTA	22		
pastrel	**PST5**	TGGCTCGTTTGGGTAGCAA	19	NEG_CONTROL	>chr3L:7345336-7345394_59bp
	PST6	CCACGACAGCAGCTTGTTGA	20		
CG17293	**MTB7**	ACAGCTATCGACCCGTCAAC	20	degenerate_E-BOX	chr2L:8374437+8374577_141bp
	MTB8	CTTGGCCACCTTAAAACTGC	20		
CG17293	**MTB55**	TTGCTTGTTCTGCCTGAATG	20	NEG CONTROL	chr2L:8368765+8368885 121bp
	MTB65	CACGTTGAAACGAACATTGG	20		
CG14621	**GP1**	CGATTACCGATTACATGCCTGAA	23	E-BOX	chrX:21845602+21845677_76bp
	GP2	ACCACATATGGACATTTGAGCAAT	24		
CG14621	**GP5**	GGCCGCTGCTCCATACAA	18	NEG_CONTROL	chrX:21839333-21839401_69bp
	GP6	CGCACTTACTATTTGCGCTTATATTT	26		
CG31436	**HK1**	CGAATTGGTGCCACCTGAGT	20	E-BOX	chr3R:21129974+21130038_65bp
	HK2	GCTCATTGCCTTCCAAAACG	20		
CG31436	**HK5**	GGGCGCCAACTGAATGTC	18	NEG_CONTROL	>chr3R:21124750-21124804_55bp
	HK6	TCCTGCATTCGAAGCCCTAT	20		
CG13248	**CAT-E1S**	GGCCACTTGAAGGATCTGAACT	22	E-BOX	>3L:20403556-20403577
	CAT-E1AS	GATATAGTCACAGCTGTTTGTGTACAACA	29		
CG13248	**137**	CAACATCAAATGCGAGATGG	20	NEG CONTROL	chr3L:20409374+20409476 103bp
	138	GCAGTTGATAAAGGCGCTTC	20		
CG32850	**EUK3**	TCGAGATTTTGCAGATGTGG	20	E-BOX	chr4:336525+336640_116bp

	EUK4	AAGGAGCAAAATTGGTCTCG	20		
CG32850	**EU5**	CATGCAGTATTGCAGCAGGT	20	NEG_CONTROL	chr4:330242+330377_136bp
	EU6	GAAACGCATCTTGGTTCTCC	20		
PHM	**E13-1F**	GATACGGGAAAAACCAGAGC	20	E-BOX	chr2R:19875488+19875627_140bp
	E13-1R	GGTTCCTGAAAAACGCCATA	20		
PHM	**UNR6-1F**	TGCAGGAGCAACACCCTTATC	21	NEG_CONTROL	6_kb_upstream,_non-coding
	UNR6-1R	CACCTGTAAGTGATCGGCGTT	21		

Table S3. Probes for 134 genes enriched > 1.5 fold change in DIMM overexpressing samples (ED34-E34) & (ED12-E12) over controls. Ordered from most to least enriched (average fold change, both developmental time points). The meaning of limma-calculated parameters is explained in the spreadsheet.

(see separate Excel File)

Table S4. Probes for 23 genes down-regulated < 0.5 fold in DIMM over expressing embryos (ED34-E34) & (ED12-E12) over controls. Ordered from most to least down-regulated (average fold change, both developmental time points). The meaning of limma calculated parameters is explained in the spreadsheet.

(see separate Excel File)

Table S5. qRT-PCR analysis of the 135 genes up-regulated by *dimm* over-expression in the embryonic CNS. 53 genes were tested and 18 showed greater than 2-fold levels following DIMM over-expression. The 11 genes selected for further study are marked in yellow. NT – not tested; ND – not detected.

EARLY+ LATE				realtime-PCR		
AvgFC	CG#	Gene Name	Gene Symbol	dimm ovx	dimm -/-	Larval CNS expression
70.1	CG12749-RA	Heterogeneous nuclear ribonucleoprotein at 85CD	Hrb87F	0.88		
55.5	CG13822-RA	---	CG13822	1.12		
32.2	Transposon.97	CG32850	---			
23.9	CG8667-RA	Dimmed	dimm	xx	0.171	Yes
17.6	CG11254-RA	maelstrom	mael	6.96	0.556	Yes
15.8	CG4577-RA	---	CG4577	NT		
14.3	CG13248-RA	---	CG13248	4.94	0.318	Yes
8.8	CG18446-RA	---	CG18446			
7.9	CG32850-RA	---	CG32850	6.15	0.519	Yes
6.7	CG12199-RC	kekkon-like	kek5	0.60		
6.3	CG8745-RA	---	CG8745	0.79		
5.4	CG33166-RB	rhomboid-2	stet	ND		
4.9	CG8023-RA	eIF4E-3	eIF4E-3	13.27	19.835	Yes
4.8	CG7785-RA	---	CG7785	4.00	0.604	Yes
4.5	CG9360-RA	chrX:11657504-11658621 (-) // 100.0 //	--- /// ---	1.27		
4.1	CG18777-RA	Cuticular protein 65Ax1 /// CG18777	Cpr65Ax1	NT		
4.1	CG15818-RA	--- /// ---	CG15818	NT		
3.9	CG32073-RA	---	CG32073	NT		
3.6	CG15093-RC	---	CG15093	NT		
3.4	CG4120-RA	Cyp12c1	Cyp12c1	NT		
3.1	CG9436-RA	---	CG9436	NT		
3.0	CG11556-RA	dm-rabphilin	Rph	ND		
2.9	CG18410-RA	---	CG18410	2.37	0.919	Yes
2.9	CG10059-RA	MAGE	MAGE	3.23	0.941	Yes
2.8	CG8407-RA	---	CG8407	NT		
2.8	CG6188-RA	---	CG6188	NT		
2.7	CG10618-RA	CHKov1	CHKov1	1.47		
2.7	CG8588-RA	pastrel	pst	2.79	0.244	Yes
2.7	CG10245-RA	Cyp6a20	Cyp6a20			
2.7	CG6522-RA	---	CG6522	3.43	0.489	Yes
2.7	CG4080-RA	---	CG4080	ND		
2.6	CT34163	gp210	gp210	NT		
2.6	CG12855-RA	HPS	HPS1	NT		
2.6	CG9684-RA	---	CG9684	NT		
2.6	CG13691-RA	BBS8	BBS8	ND		
2.5	CG13679-RB	---	CG13679	NT		
2.5	CG3832-RA	Peptidyl gycine alpha hydroxylating monooxygenase	Phm	3.16	0.678	Yes
2.5	CG5322-RA	---	CG5322	NT		
2.5	CG14621-RA	---	CG14621	3.05	0.563	Yes
2.4	CG31436-RA	---	CG31436	6.15	0.768	Yes

2.3	**CG1275-RB**	**---**	**CG1275**	2.91	0.418	Yes
2.3	**CG8729-RA**	**ribonuclease H1**	**rnh1**	**NT**		
2.3	**CG33207-RA**	**baikal**	**pxb**	**NT**		
2.3	**CG8097-RA**	**---**	**CG8097**	2.21	0.932	Yes
2.3	**CG33126-RA**	**Neural Lazarillo**	**NLaz**	**NT**		
2.3	**CG9990-RA**	**---**	**CG9990**	**NT**		
2.3	**CG3570-RA**	**---**	**CG3570**	1.87		
2.2	**CG13333-RA**	**---**	**CG13333**	**NT**		
2.2	**CG8353-RA**	**---**	**CG8353**	**NT**		
2.2	**CG30420-RA**	**Activating transcription factor-2**	**Atf-2**	**NT**		
2.2	**CG10242-RA**	**Cyp6a23**	**Cyp6a23**	**NT**		
2.2	**CG5105-RA**	**Phospholipase A2 activator protein**	**Plap**	**NT**		
2.1	**CG3832-RB**	**Peptidyl gycine alpha hydroxylating monooxygenase**	**Phm**	**NT**		
2.1	**CG6236-RB**	**---**	**CG6236**	0.94		
2.1	**CG14933-RA**	**--- /// CG42501**	**CG14933**	**NT**		
2.1	**CG6167-RA**	**PICK1**	**PICK1**	**NT**		
2.1	**CG14021-RB**	**CG14021**	**fusl**	0.35		
2.1	**CG2916-RB**	**septin**	**septin**	**NT**		
2.0	**CG10248-RA**	**cytochrome P450 related AF5**	**Cyp6a8**	1.31		
2.0	**CG5300-RA**	**Klp31E**	**Klp31E**	0.91		
2.0	**CG8629-RA**	**---**	**CG8629**	**NT**		
2.0	**CG7321-RA**	**Rim**	**Rim**	**NT**		
2.0	**CG3603-RA**	**chrX:2842068-2842983 (+) // 100.0 //**	**---**	**NT**		
2.0	**CG9092-RA**	**beta-galactosidase-1**	**Gal**	**NT**		
2.0	**CG14375-RA**	**---**	**CG14375**	**NT**		
2.0	**CG16926-RA**	**---**	**CG16926**	**NT**		
2.0	**CG7224-RA**	**---**	**CG7224**	1.25		
2.0	**CG30269-RA**	**---**	**CG30269**	1.25		
1.9	**CG7940-RA**	**CG7940**	**Arp5**	**NT**		
1.9	**CG8202-RA**	**---**	**CG8202**	**NT**		
1.9	**CG6981-RB**	**---**	**CG6981**	1.87		
1.9	**CG31688-RA**	**---**	**CG31688**	**NT**		
1.9	**CG17293-RA**	**---**	**CG17293**	2.00	0.659	Yes
1.9	**CG14512-RA**	**---**	**CG14512**	**NT**		
1.8	**CG8960-RA**	**---**	**CG8960**	2.19	NT	
1.8	**CG6438-RA**	**amontillado**	**amon**	**NT**		
1.8	**CG33300-RA**	**Mucin 30E**	**Muc30E**	**NT**		
1.8	**CG1615-RB**	**Open rectifier K[+] channel 1**	**Ork1**	**NT**		
1.8	**CG4686-RA**	**--- /// GA18356**	**CG4686**	**NT**		
1.8	**CG3380-RA**	**Organic anion transporting polypeptide 58Dc**	**Oatp58Dc**	**NT**		
1.8	**CG10638-RA**	**---**	**CG10638**	2.12	NT	
1.8	**CG17632-RA**	**Plum**	**bw**	**NT**		
1.8	**CG12926-RA**	**---**	**CG12926**	**NT**		
1.8	**CG12746-RA**	**---**	**CG12746**	**NT**		
1.8	**CG13678-RA**	**---**	**CG13678**	**NT**		
1.8	**CG11328-RA**	**Nhe3**	**Nhe3**	1.30		
1.8	**CG12659-RB**	**--- /// ---**	**CG12659**	**NT**		

1.8	CG4859-RB	Matrix metalloproteinase 1	Mmp1	NT
1.8	CG4753-RB	---	CG4753	NT
1.8	CG12367-RA	CG12367	Pimet	NT
1.7	CG10679-RA	Nedd8	Nedd8	NT
1.7	CG2093-RA	CG2093	Vps13	NT
1.7	CG16738-RA	---	March 2006 (FlyBase/BDGP 5.3)	NT
1.7	CG5063-RB	Translin associated factor X	Trax	NT
1.7	CG2890-RA	Protein phosphatase 4 regulatory subunit 2-related protein	PPP4R2r	NT
1.7	CG5486-RA	Ubiquitin-specific protease 64E	Ubp64E	0.91
1.7	CG9331-RB	chr2L:20824435-20827744 (+) // 100.0 //	---	NT
1.7	CG11584-RB	---	CG11584	NT
1.7	CG32452-RA	---	CG32452	NT
1.7	CG10354-RA	CG10354	Rat1	NT
1.7	CG6119-RB	Vesicular monoamine transporter	Vmat	NT
1.7	CG4799-RA	March 2006 (FlyBase/BDGP 5.3)	chr2L:10056905-10060097 (+) // 100.0 //	NT
1.7	CG5492-RA	Tetraspanin 74F	Tsp74F	NT
1.7	CG31683-RA	---	CG31683	NT
1.7	CG32120-RA	senseless	sens	NT
1.7	CG3931-RA	Rrp4	Rrp4	NT
1.7	CG31617-RA	His1:CG31617 ///	His1:CG31617	NT
1.6	CG11205-RA	--- /// photolyase	CG18853 /// phr	NT
1.6	CG10371-RB	MKP-like	Plip	NT
1.6	CG7865-RB	PNGase	PNGase	NT
1.6	CG1009-RD	Puromycin sensitive aminopeptidase	Psa	1.35
1.6	CG9226-RA	WD repeat domain 79 homolog	WDR79	NT
1.6	CG15706-RA	---	CG15706	NT
1.6	CG12143-RA	CG12143	Tsp42Ej	NT
1.6	CG1233-RB	---	CG1233	NT
1.6	CG32317-RB	---	---	NT
1.6	CG10732-RA	---	CG10732	NT
1.6	CG10042-RB	MBD-R2	MBD-R2	NT
1.6	CG14696-RA	--- /// --- /// ---	CG14696	NT
1.6	CG31155-RA	chr3R:11048121-11048814 (-) // 100.0 //	Rpb7	NT
1.6	CG31650-RA	---	CG31650	NT
1.6	CG2957-RA	mitochondrial ribosomal protein S9	mRpS9	NT
1.6	CG33047-RA	--- /// alpha-L-fucosidase	Fuca	NT
1.6	CG30101-RA	---	CG30101	NT
1.6	CG14057-RA	---	CG14057	NT
1.6	CG18600-RA	---	CG18600	NT
1.6	CG9900-RB	Zeste-White 10	mit(1)15	NT
1.6	CG5930-RC	Transcription factor IIA L	TfIIA-L	NT
1.6	CG31109-RA	---	CG31109	NT
1.6	CG12797-RA	Ciao1	Ciao1	NT

1.6	**CG10424-RA**	**---**	**CG10424**	**NT**
1.6	**CG6550-RA**	**---**	**CG6550**	**NT**
1.5	**CG7903-RA**	**---**	**CG7903**	**NT**
1.5	**CG16784-RC**	**purple**	**pr**	**NT**
1.5	**CG7161-RA**	**Oseg1**	**Oseg1**	**NT**
1.5	**CG32795-RA**	**---**	**CG32795**	**NT**
1.5	**CG31118-RA**	**RabX4**	**RabX4**	**NT**

Table S6. The eleven candidate DIMM-regulated genes – identification, mammalian orthologues and GO annotations.

Gene	Homologue	Gene Ontology
CG3832 (PHM)	**PAM**	monooxygenase; peptide amidation; secretory vesicle
CG1275	**Cybrd1**	Cyt b-561; electron carrier activity; transport vesicle
CG13248 (CAT-4)	**SLC7A4**	Cationic amino acid transmembrane transporter
CG11254 (mael)	**MAEL**	HMG box; cytoskeleton organization
CG17293	**wdr82**	WD40 superfamily; COMPASS complex subunit
CG7785	**CLLD6**	SPRY superfamily
CG6522	**Dyxin/Testin**	LIM domain; PET domain; Transcriptional cofactor
CG32850	**RNF11**	Ring finger domain; Interact with HECT-type Ubiquitin Liga
CG14621	**SLC35E1**	glucose 6-phosphate:phosphate antiporter; membrane
CG31436		Protein kinase c-like superfamily;
CG8588 (pst)		learning or memory; olfactory learning; cytosol

Table S7. Probes for 535 genes enriched > 1.5 fold change and satisfying a 5% False Discovery Rate in large LNvs versus small LNvs. Ordered from most to least enriched (average fold change). The meaning of limma-calculated parameters is explained in the spreadsheet.

(see separate Excel File)

Table S8. Probes for 537 genes enriched > 1.5 fold change and satisfying a 5% False Discovery Rate in small LNvs versus large LNvs. Ordered from most to least enriched (average fold change). The meaning of limma-calculated parameters is explained in the spreadsheet.

(see separate Excel File)

Experimental Procedures

Fly stocks. The following fly lines were used: *dimm* deletion mutant alleles (*Rev4* and *Rev8*); UAS-*dimm*-myc (II or III) [1]; UAS-*ppMII* [2], UAS-*2X EGFP* and the pan-neuronal driver, *elav*-GAL4 (III), *GMR*-GAL4 (II). For quantitative mass spectrometry, we used UAS-RNAi transgenic lines obtained from the Vienna RNAi stock center (VDRC) to knockdown target genes (See Supplemental Information), combined with UAS-*DCR2* (obtained from Stefan Thor, Linkoping Univ., Sweden). The following strains were used for quantitative mass spectrometry:

w, UAS-*DCR2;pGMR*-GAL4 *(II);* UAS-*ppMII (III)*

w; UAS-*dimm-myc (II or III)*;UAS- *dsRNAi* lines (II or III).

The following strains were used for ChIP analysis**:**

w^{1118}*; c929*-GAL4/UAS-*dimm-myc (II);;tub*-GAL80^{ts}

w^{1118}*; c929*-GAL4*;tub*-GAL80^{ts} (as a negative control strain)

Microarrays. We combined UAS-*dimm-myc* (II) with *elav*-GAL4; for microarray analysis, these were compared to *elav*-GAL4. Crosses were maintained 18^{o}C to minimize lethality. RNA was prepared from embryos collected at 22-26 hr (~ embryo Stage 14) and at 28-32 hr (~ embryo Stage 16). A pair of GeneChip® *Drosophila* Genome 2.0 arrays (Affymetrix Co., Santa Clara, CA) were tested with each RNA sample (two experimental and two control, for each of two time points). Eight microarrays were used to hybridize cDNAs from experimental *dimm::myc*-expressing

and control flies, sampled at two different embryonic stages. Four microarrays were hybridized with cDNA from the *dimm::myc*-expressing embryos of genotype w;UAS-*dimm-myc* (II)/+;*elav*-GAL4/+; (prefix "ED"). Another four microarrays were hybridized with cDNAs from the parental control strain: w;;*elav*-GAL4 (prefix "E"). Samples ED3 and ED4, E3 and E4 were hybridized with cDNA from stage 14 embryos (24-26hr development). Samples ED1 and ED2, E1 and E2 were hybridized with cDNA from stage 16 embryos (28-32 hr old embryos). A pair of GeneChip® Drosophila Genome 2.0 arrays (Affymetrix Co., Santa Clara, CA) were tested with each RNA sample (two experimental and two control, for each of two time points). All microarray data are publically available (GEO accession #GSE31113).

Affymetrix CEL files raw probe intensity data was loaded into the statistical computing language R v. 2.12.1 for Snow Leopard (32-bit build) with the affy / simpleaffy libraries (ReadAffy). Microarrays were quality checked with the affyPLM and affycoretools libraries. Probe intensities were then $\log_2$ normalized with the gcrma algorithm version 2.22.0 (default settings). All eight arrays were gcrma-normalized together as one data set. Gcrma-normalized data sets were then processed with the limma package (Linear Models for Microarray Data) v. 3.6.9 and default limma settings [3]. Contrast matrices were set up to look for probes that were differentially expressed in the ED samples compared to E samples for each age: (ED3,ED4)-(E3,E4) and (ED1,ED2)-(E1,E2).

Limma comparison results are displayed in terms of linear scale fold changes when ED probes are compared to E probes. The ED34-E34 comparison yielded 360 probes that were at least 1.5 fold up-regulated. There were 48 probes that were below 0.5 fold in the ED34-E34 comparison, indicating down-regulation. The ED12-E12 comparison yielded 342 ED probes that were at least 1.5 fold above E probes and 97 ED probes below 0.5 fold of E probes.

In order to correct for multiple comparisons, Benjamini-Hochberg False Discovery Rate (FDR) corrections implemented in limma were used. There were 880 and 912 differentially expressed genes with conventional p-values < 0.05 in the ED34-E34 and ED12-E12 comparisons, respectively. Of these, 21 / 880 and 44 / 912 satisfied a 5% FDR cutoff. Failure to obtain a higher number of significant hits after Benjamini-Hochberg correction at 5% FDR was likely related to the relatively small number of replicates (two) in the experiment. Furthermore, sampling whole embryos as opposed to only *elav*-positive cells yielded a low signal to noise ratio.

We therefore decided to use the 1.5 fold enrichment cutoff in identifying differentially expressed genes. We first sought to identify overlapping probes that were above 1.5 fold enriched in both the early and late embryos. To obtain the intersection of these probe sets, queries were run in Galaxy, an open platform for genomic research [4-5]. Galaxy queries were setup to find which of the 360 ED34-E34 enriched probes overlapped with the 342 ED12-E12 enriched probes. This intersection yielded a list of 136 probes (135 genes, Supplementary Table 3). We also identified overlap between the

48 probes below 0.5-fold in ED34-E34 and the 98 probes in ED12-E12. This intersection included 22 probes that were downregulated in both early and late embryos at 0.5-fold level or less (Supplementary Table 4).

We compared the list of DIMM overexpression-enriched genes generated in this study with the data generated by Kula-Eversole *et al.* [6] obtained from purified "normal" DIMM neurons (adult LNv). In order to directly compare our study with theirs, we reanalyzed the raw data from [6], with the same methods used for the embryo data. Additionally, Kula-Eversole *et al*. compared large LNv and small LNv probe data to *elav* cells sat different circadian time points. We wanted to compare large LNv neurons directly to small LNv neurons regardless of circadian time. For these reasons, data from Kula-Eversole *et al.* were reanalyzed as follows: all CEL files from the study were obtained from the Gene Expression Omnibus depository under the accession number GSE22308. The 24 CELs were gcrma-normalized together followed by limma analysis of probes enriched in Large-Small LNv neurons.

We analyzed all Large LNv (10 samples) and small LNv (4 samples) files regardless of the circadian time when RNAs from purified LNv neurons were harvested. For this analysis, we assumed that DIMM is not involved directly in the circadian system and that therefore those genes that DIMM activates directly should be enriched in Large LNv neurons across circadian time points. Limma was used to identify differentially expressed probes in the Large LNv-small LNv comparison at a Benjamini-Hochberg FDR of 5% and a 1.5 fold minimum fold enrichment. 535 probes were 1.5 fold enriched

in Large LNvs over small LNvs with a 5% FDR (Supplementary Table 7). 537 Large LNv probes were below 0.5 fold of small LNv with a 5% FDR (Supplementary Table 8).

Next, the goal was to identify overlapping probe sets that are enriched upon embryonic DIMM overexpression and in normal adult DIMM-positive Large LNv neurons when compared to DIMM-negative small LNvs. Galaxy queries were run to find the intersection of 136 enriched probes from the embryo and the 535 Large LNv-enriched probes from the adult. The intersection of these data sets yielded a set of 18 genes (Figure 7; Supplementary Table 3). There were no overlapping hits when 22 probes that were <0.5 fold in both embryonic samples (Supplementary Table 4) were compared to probes that are <0.5 fold and 5% FDR in the Large-small comparison (Supplementary Table 8). The term DIMM-negative is used as a descriptor and is not meant to suggest that the small LNv have no DIMM expression.

qPCR analyses. To evaluate candidate genes, quantitative real time-PCR (qPCR) was performed using RNA derived from 24-32hr embryos collected at 18^{o}C and mis-expressing either DIMM (UAS-*dimm*) or CD8-EGFP (UAS-*CD8-EGFP*) driven by *elav*-GAL4. To analyze *dimm* mutants, first instar larvae trans-heterozygous for two *dimm* alleles (*rev4/rev8* – [1]), or first instar larvae from the control w^{1118}, were collected at RT. To analyze neural RNAs, one hundred third instar larval CNS of w^{1118} were manually dissected. Total RNA was isolated with Trizol reagent (Sigma, St Louis, MO), digested with RNase-free DNase I, and purified with RNAeasy columns (Qiagen, Madison, WI). Reverse transcription reactions were performed following the manufacturer's protocols

(NEB). We measured transcript quantities using SYBR-green incorporation on an ABI 7000 machine and made genotypic comparisons with the double delta Ct method [7]. Levels of *RP49* RNA were used for normalization control. The primers used are listed in Supplementary Table 1.

***In situ* hybridizations and antibody staining of embryos.** We followed general methods previously described [8]. Clones containing the cDNA of interest were obtained from the PCR of the cDNA template. For probe synthesis, plasmids were linearized with appropriate restriction enzymes at the 5' end of the coding sequence. Transcription reactions used the digU RNA labeling mix (Roche, final concentrations 1mM each ATP, CTP and GTP, 0.65 mM UTP, 0.35 mM DIG-11-UTP) and the appropriate RNA polymerase (NEB, Roche, or Promega) for 2 hrs at 37^{o}C. Probes were then hydrolyzed in carbonate buffer (final concentration 60 mM Na_2CO_3, 40mM $NaHCO_3$, pH 10.2) for 40 minutes at 65^{o}C. The reactions were neutralized with stop solution (final concentration 0.1 M sodium acetate pH 6.0), and the RNA is precipitated with LiCl and re-suspended in 150 ml hybridization solution (see below). Typically, between 2 and 4 ul of probe was used in a 100 ml hybridization reaction.

Embryos were collected and aged on yeasted grape plates. Due to embryonic lethality at room temperature, for the *dimm* overexpression studies all collections and aging was done at 18^{o}C. Embryos were dechorionated in 50% bleach and fixed in 50:50 heptane: embryo fix buffer (1xPBS, 50 mM EGTA pH 8.0, 10% formaldehyde) for 25 minutes with vigorous agitation, followed by devitellinization with MeOH. The embryos

were cleared using Xylenes, postfixed in 5% formaldehyde in PBT (1xPBS + 0.1% Tween 20) and then treated with proteinase K (Roche, 4 ug/ml in PBT) for 10 minutes. This was followed by another postfix and prehybridization for 1 hr in hybridization solution (50% de-ionized formamide (American Bioanalytical), 5x SSC, 100 ug/ml sonicated, boiled salmon sperm DNA (Sigma), 50 ug/ml heparin (Sigma), 0.1% Tween 20 (Fisher)) at 55^{o}C. The embryos were then hybridized with antisense RNA probes in hybridization solution for 18 hrs at 55^{o}C. After washing with hybridization solution at 55^{o}C and PBT at room temperature, the embryos were incubated with alkaline phosphatase labeled anti-digoxygenin antibody (Fab fragments, Roche, pre-absorbed, diluted at 1:2,000) overnight at 4^{o}C. After washing with PBT, the staining was developed with 4-nitro blue tetrazolium (NBT, Roche, 0.675mg/ml) and X-phosphate (Roche, 0.35mg/ml) in AP staining buffer (100mM NaCl, 50mM MgCl2, 100mM Tris pH 9.5, 0.1% Tween 20). Embryos were mounted in permount (Fisher) or 70% glycerol in PBS and viewed using a Zeiss Axioplan2 microscope. Images were obtained with an Olympus DP71 camera and manufacturer's software.

Transactivation. Assays followed the general methods previously described [9]; Genomic fragments from the loci encoding candidate genes were subcloned into the *pGL3* vector (Promega, Madison WI). *Drosophila BG3-c2* neuronal cells (1x 10^{6} cells per well) were transiently transfected with a total 1.5μg of DNA by mixing with 5 ul of FuGENE^{HD} (Roche) and incubating for two days at RT. Transactivation was measured by a Luciferase assay system (Promega, Madison, WI). For each experiment, a vector containing *pActin-LacZ* was co-transfected to normalize the transfection efficiency, and

each transfection was performed at least three times independently. The significance was tested by the student's T-test (two tailed).

Immunocytochemistry and Imaging. We generated antibodies and used immunostaining methods as previously described [1, 10, 11]. Affinity purified guinea pig anti-DIMM (1:200; [10]), rabbit anti-FMRFa (1:1000; [11]), mouse monoclonal anti-GFP 3E6 (1:800, Molecular Probes, Carlsbad CA) and rabbit anti-GFP (1:500; rabbit polyclonal, #AB3080 Chemicon, Temecula, CA) were used as primary antibodies. Cy3-conjugated- (Jackson Immunoresearch, West Grove, PA) or Alex-488-conjugated- (Molecular Probes, Carlsbad CA) secondary antibodies were used for immunocytochemistry. To generate an antibody against the CG13248 protein product, we constructed a GST-fusion containing the predicted C-terminal (cytoplasmic) region of the protein (596-669 A.A.; 75 amino acids) and produced GST-CAT-4 in *E. coli*. Guinea pig anti-CAT-4 protein was used for immunostaining, using methods previously described [1, 10, 11]. Confocal images were acquired on an Olympus FV500 laser scanning confocal microscope and manipulated by ImageJ and Adobe Photoshop software to adjust contrast.

Chromatin Immunoprecipitation (ChIP) analysis. ChIP was performed as described by Menet *et al.* [12]. w^{1118}; *c929*-GAL4;*tub*-GAL80^{ts} females were crossed to y^1w^1; UAS-*dimm-myc* (II) males at 18^{o}C. To control for non-specific antibody binding, non-specific DNA enrichment (through binding to sepharose beads, reaction surfaces etc. [13]), ChIPs were also performed on the w^{1118}*; c929*-GAL4; *tub*-GAL80^{ts} parental strain that lacks the *dimm::myc* transgene. Progeny developed at 18^{o}C through adult eclosion.

Two to five day old adults of the following two genotypes (y^1 w^1/ +; *c929*-GAL4/UAS-*dimm-myc* (II); *tub*-GAL80^{ts}/+ and w^{1118}; *c929*-GAL4; *tub*-GAL80^{ts}) were shifted to 30^{o}C for 3 days. Flies were collected on dry ice, and heads were sieved the following day through 25 and 40 micron sieves. One milliliter of fly heads was homogenized in 7 ml Wheaton-Dounce homogenizers with the type B (tight) pestle in 3 mL of NEB buffer (10 mM HEPES-Na at pH 8.0, 10 mM NaCl, 0.1 mM EGTA-Na at pH 8.0, 0.5 mM EDTA-Na at pH 8.0, 1 mM DTT, 0.5% Tergitol NP-10, 0.5 mM Spermidine, 0.15 mM Spermine plus protease inhibitor cocktail [Roche mini]) for a total of 30 min (2-min homogenization 10 times, 1 min on ice 10 times). Homogenate was dumped into a 70-mm cell strainer placed in a 50-mL falcon tube and centrifuged at 300g for 2 min. Filtered homogenate was split into three fractions, transferred to microfuge tubes and centrifuged at 6,000 x g for 10 min. The nuclei-containing pellets were resuspended in 1 mL of NEB and centrifuged at 20,000 x g for 20 min on sucrose gradient (0.65 mL of 1.6 M sucrose in NEB, 0.35 mL of 0.8 M sucrose in NEB). The pellet was resuspended in 1 mL of NEB and 11% formaldehyde (diluted in Schneider's media; Sigma) was added to a final concentration of 1%. Nuclei were cross-linked for 10 min at room temperature on a rotator before cross-linking was quenched by adding 1/10 vol of 1.375 M glycine for 5 min. The nuclei were collected by centrifugation at 6,000 x g for 5 min. All centrifugations steps were carried out at 4^{o}C. Nuclei were washed twice in 1 mL of NEB and resuspended in 450 mL of Sonication buffer (10 mM HEPES-Na at pH 7.5, 2 mM EDTA at pH 8.0, 1% SDS, 0.2% Trition X-100, 0.5 mM Spermidine, 0.15 mM Spermine plus Roche mini protease inhibitor cocktail). Two biological replicates of nuclei were sonicated using a Fisherbrand Sonic Dismembranator at setting 2 (57 W) five times for

15 sec on and 2 min off (set on ice). Three other biological replicates were sonicated on a Misonix 3000 sonicator fitted with a microtip at setting P=5.0 (12-15W) 6 - 12 times for 15 sec on and 1 minute off (set in ice-ethanol bath). Sonicated nuclei were centrifuged at 15,000 x g for 10 min and frozen at -80°C in 150 µl aliquots. The majority of the sonicated chromatin had a unimodal sequence length distribution with a peak around 500 base pairs. Twenty-five microliters of sonicated chromatin were removed for the input sample. The remaining 125µl of chromatin was diluted in 1.1 mL of IP buffer (50 mM HEPES/KOH at pH 7.6, 2 mM EDTA, 1% Triton, 0.1% NaDeoxycholate in PBS). Samples were rotated overnight at 4°C after adding antibodies: 15 µl of anti-c-MYC tag goat antibody (Abcam ab9132). Protein G-Sepharose beads (Zymed) or protein G-sepharose beads (Sigma, Saint Louis, MO) were blocked overnight in 0.1 mg/mL yeast tRNA and 1 mg/mL BSA in IP buffer. After overnight incubation, the beads were washed once in IP buffer, added to the chromatin/antibody mixture, and then incubated for an additional 2 h at 4°C.

Beads were spun down at 10,000 rpm for 20 sec and were washed once in 1.5 mL of ChIP Wash buffer (50 mM HEPES-KOH at pH 7.6, 1 mM EDTA, 1% Triton, 0.1% NaDeoxycholate, 0.1% Sarkosyl, 0.1% BSA, 0.5 M KCl in PBS). Beads were resuspended in 1 mL of ChIP Wash buffer and rotated for 30 min at 4°C. Beads were then washed once in Li Wash Buffer (10 mM Tris-Cl at pH 8.0, 0.25 M LiCl, 0.5% NP40, 0.5% NaDeooxychoalte, 1 mM EDTA) and once in cold TE (pH 8.0) before being eluted with 150 mL of ChIP Elution buffer (50 mM Tris-HCl at pH 8.0, 10 mM EDTA, 1% SDS, 1 mM DTT, 0.1 mg/mL Proteinase K). ChIP Elution buffer (150 mL) was also

added to the input sample. Both IP and input samples were incubated for 2 h at 37°C. The sepharose beads were removed from the IP samples and then all samples were decrosslinked overnight at 65°C. DNA was isolated from the samples using PCR purification kit (Qiagen).

Real-time quantitative PCR (qPCR) following ChIP. The PCR mixture contained Platinum Taq polymerase (Invitrogen) and optimized concentrations of Sybr-Green (Invitrogen). The sequences of primers used are listed in Table S2qPCR was performed in triplicate on at least two, in most cases three independent biological replicates of chromatin obtained from different sets of fly heads on different days. For each primer set, 1:10 dilutions of ChIP and input DNA from both strains were prepared from the same master mix. Additionally, for each primer set, one of the inputs was further diluted to make 1:100 and 1:1,000 dilutions. This was necessary to create calibration curves to ensure reaction linearity. Fluorescence intensities were plotted versus the number of cycles by using an algorithm provided by Corbett Research (Qiagen). For each gene, at least two primer sets were designed. Only reactions whose R^2 values were > 0.99 were considered valid. Real-time qPCR was performed a Corbett Research Rotor-Gene 3000 real-time cycler or a RotorGene Q real-time PCR machine with cycling parameters: 3 min at 95°C, followed by 40 cycles of 30 sec at 95°C, 45 sec at 55°C, and 45 sec at 72°C. One set amplified regions around or immediately adjacent to CANNTG canonical E-boxes in genes' first introns or first exons / 5' UTR. A second set amplified genomic regions approximately 6 kilobases upstream of E-box primer sets. Each ChIP was normalized to its input by delta-Ct value. Delta-delta-Ct value was then calculated by

subtracting delta-Ct of negative control from delta-Ct of the tagged strain. The fold-difference between experimental and control samples was obtained by 2^(-delta-deltaCt). The properties of the primers used are listed in Supplementary Table 2.

Quantitative Mass Spectrometry. Fly heads of the control (*GMR*>UAS-*ppMII*; UAS-*dimm*) and experimental (*GMR*>UAS-*ppMII*; UAS-*dimm*; UAS-RNAi) transgenic lines were collected in frozen state. MS-based quantitation was based on prior quantitative measurement approaches [14-16]. Frozen heads were homogenized in acidified acetone (40:6:1, acetone:water:concentrated HCl, v/v/v; 10 ul/head). The homogenate was centrifuged at 12,000 rpm for 5 min: supernatants were concentrated by drying in a SpeedVac (Thermo Electron Co.), and then reconstituted with 2% acetonitrile aqueous solution (containing 0.1% FA and 0.01% TFA) for subsequent analysis. The quantitative analysis on MII fully processed from ppMII was performed using capillary liquid chromatography coupled to matrix-assisted laser/desorption ionization time-of-flight mass spectrometry (CapLC-MALDI-TOF/TOF MS). We performed relative quantitation using two approaches. For the labeling approach, the samples were labeled with succinic anhydride as previous described [14-15]. Briefly, 2 µl of 2 M light and heavy forms of succinic anhydride (Sigma Aldrich) in DMSO were used to label the control and experimental samples, and the sample solutions were adjusted to be basic. After the reaction, the labeled control and experimental samples were combined before being desalted using spin columns (Pierce, Rockford, IL). The combined and desalted samples were then analyzed with CapLC-MALDI-TOF/TOF MS (Bruker Daltonics, Billerica, MA). 5 µl samples were separated with a reverse phase column (Alltech

Associates Inc., Alltima HP C18, 150 mm × 300 um, 3 μm particle diameter, 100 Å pore size) at a flow rate of 2 μl/min. Solvent A contains 95% water, 5% acetonitrile, 0.1% FA, and 0.01% TFA, and Solvent B contains 5% water, 95% acetonitrile, 0.1% FA, and 0.01% TFA. The 45-min gradient started from 2% B to 20% B over 10 min, to 40% B in another 15 min, continued to 80% B in 7 min, and stayed at 80% B for 3 min before ramping back to 2% B. The fraction collection started at 20 min, and a total of 24 one-minute fractions were collected on a MALDI target for each sample. 1 μl of *a*-cyano-4-hydroxycinnamic acid (Sigma Aldrich) in 70/30 (v/v) acetonitrile/ water solution was used as matrix, and MALDI-TOF/TOF mass spectra were acquired in the mass range of 600-5000 Da. The relative peak intensities in the peak pair were used to indicate the relative amount of peptides between control and experimental samples.

In the label-free quantitation approach, *Drosophila* head extracts were analyzed with CapLC-MALDI-TOF/TOF MS described earlier just after they were reconstituted. Endogenous peptide Drm-MT2 (hugin (*CG6371*) (m/z 942.59) and exogenous peptide MII (m/z 1710.69) have been well studied in *Drosophila* previously [2, 17], and their presence was determined by mass matching between the theoretical masses and the experimental masses at signal-to-noise ratio above 3 within 20 ppm. MT2 and MII were respectively isolated with MALDI-TOF/TOF tandem MS, and the intensity of the isolated parent ion was used to reflect the amount of the peptide after normalization. Student's t-test was used for statistics with at least three biological replicates.

RNAi transgenic lines used in this study.

CG#	GENE	RNAi line
CG13248	*CAT-4*	GD5384 (VDRC)
CG1275	*Cyt-b$_{561-1}$*	GD4103 (VDRC)
CG11254	*mael*	GD18198 (VDRC)
CG7785	*cddl6*	GD36650 (VDRC)
CG32850	*rnf11*	JF01121 (Harvard)
CG6522		GD22500(VDRC)
CG14321		GD29814 (VDRC)
CG17293	*wdr82*	GD25246 (VDRC)
CG31436		GD21341 (VDRC)
CG14621		GD8661 (VDRC)

Supplemental References

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