Cell Reports, Volume *39*

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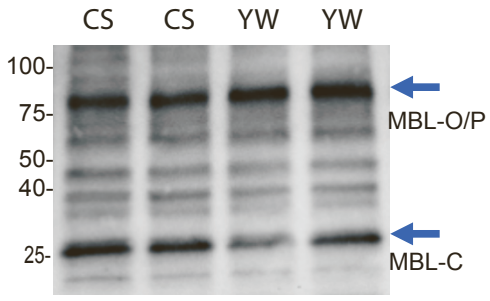
A.

Isoform	RNAseq	RFP
mbl-A	0.06	0
mbl-B	0.05	0
mbl-Mi	0.1	0.05
mbl-C/O/P	0.77	0.95

B.

Coordinates of junction	Isoform	RNAseq	RFP
chr2R: 17371153-17373077	*mbl-O/P*	74	72
chr2R: 17362097-17373077	*mbl-C*	72	77
chr2R: 17373161-17373818	*mbl-C/O/P*	150	68

C.



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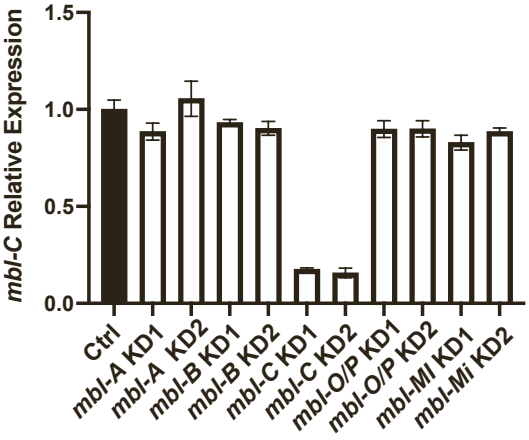

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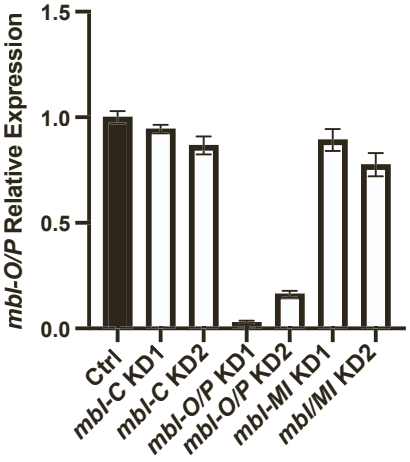
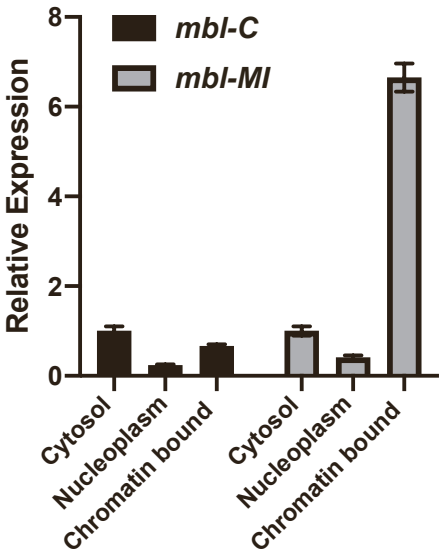


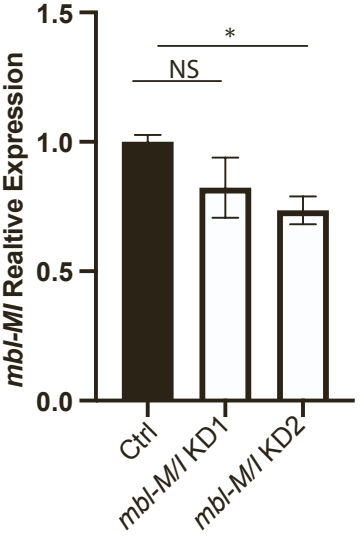
D.

Fly Strain	Viability (% Control)
Mbl-A KD1	101
Mbl-A KD2	94
Mbl-B KD1	96
Mbl-B KD2	98
Mbl-C KD1	105
Mbl-C KD2	112
Mbl-O/P KD1	100
Mbl-O/P KD2	79
Mbl-M/I KD1	112
Mbl-M/I KD2	92
UTR KD1	12
UTR KD2	113
E1 KD1	82
E1 KD2	107
E2 KD1	0
E2 KD2	0
E2-3 KD1	0
E2-3 KD2	0

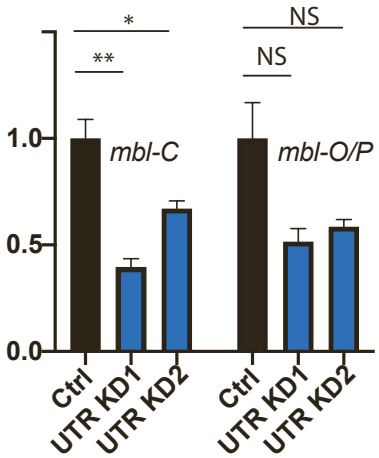
G.



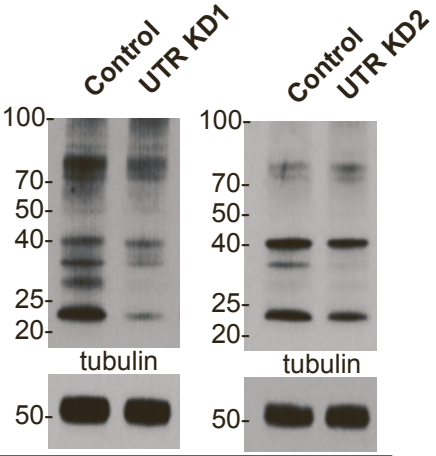
H.



I.



J.



MBL-C	1.00	0.13		1.00	0.62
MBL-O/P	1.00	0.83		1.00	1.05

Figure S1.

Figure S1 related to Figure 1: Mbl locus generates several RNA and protein isoforms and can be knockdown specifically using isoform specific shRNAs. A, Proportion of reads aligning to each *mbl* 3'UTR normalized by length. **B.** Number of junction reads of *mbl-O/P* and/or *mbl-C* isoforms detected in RNA sequencing and RFP reads. **C.** Western blot analysis of fly heads extract from CS and *YW* flies with anti-MBL, tubulin is blotted as a loading control. **D.** Viability count for different *mbl* isoform KD lines % was plotted against control sibling flies. **E**, **F.** qRT-PCR evaluation of Relative *mbl-C* and *mbl-O/*P expression levels in different *mbl* isoform KD lines respectively. **G.** qRT-PCR evaluation of *mbl-C* and *mbl-M/I* expression levels at various cellular fractions from fly heads. **H.** qRT-PCR evaluation of *mbl-M/I* relative expression in *mbl-M/I* KD flies. **I.** qRT-PCR evaluation of Relative *mbl-C* and *mbl-O/P* expression levels in UTR KD lines respectively. **J.** Western blot analysis of fly heads extract from control and UTR KD flies with anti-Mbl, tubulin is blotted as a loading control. In all the qRT-PCR analysis tubulin was used as a normalization control (n = 3, error bars represent standard error of the mean, two-tailed t.test performed for significance difference (****, $p<0.0001$, ***, $p<0.0002$, **, $p<0.0021$, *, $p<0.0332$)).

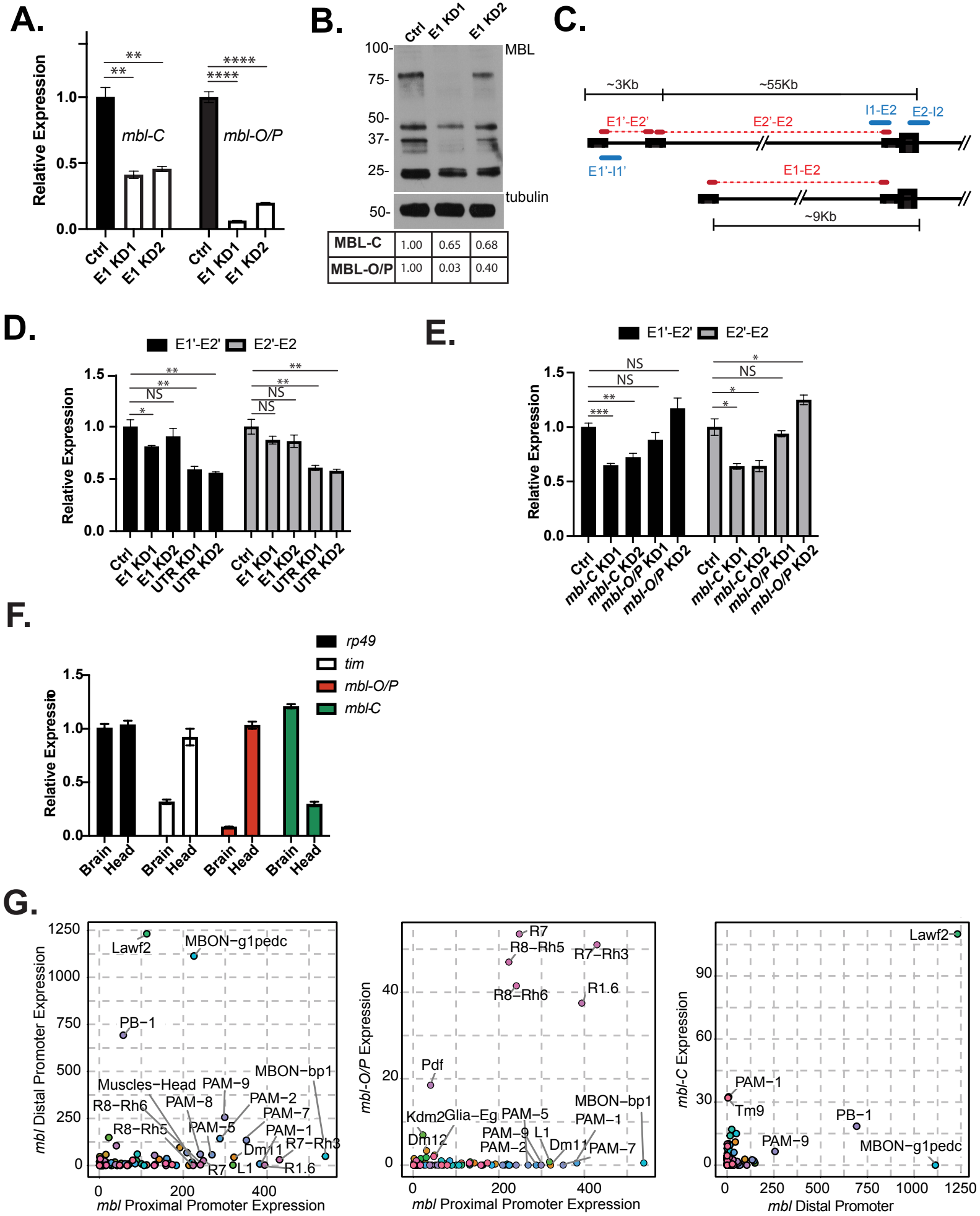
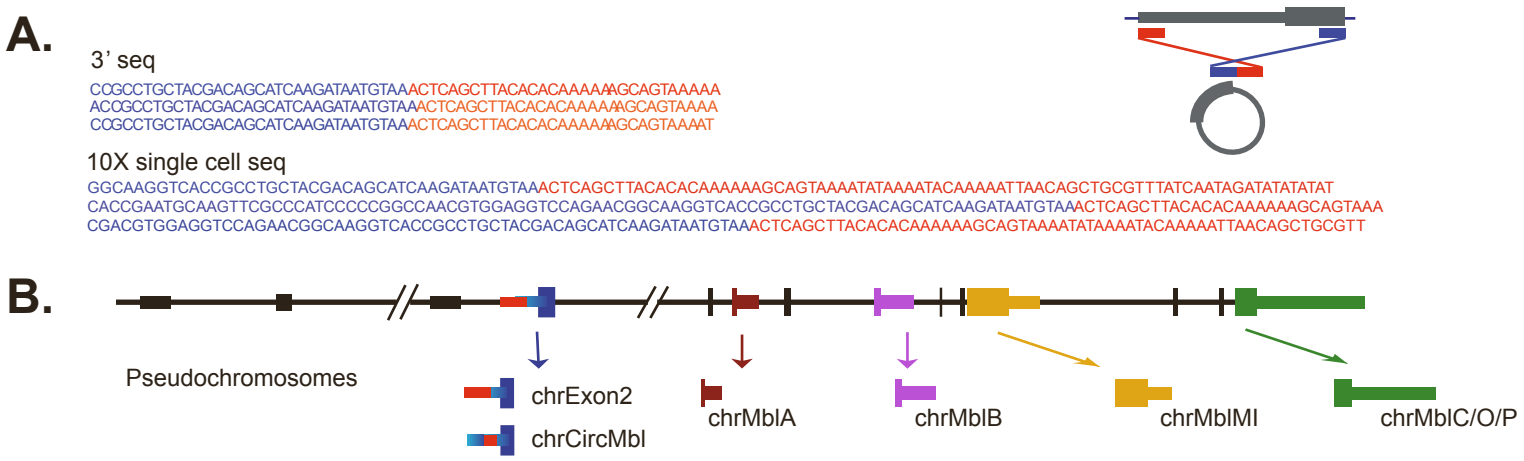

A.
Relative Expression
mbl-C
mbl-O/P
Ctrl
E1 KD1
E1 KD2
B.
Ctrl
E1 KD1
E1 KD2
MBL
tubulin
MBL-C 1.00 0.65 0.68
MBL-O/P 1.00 0.03 0.40
C.
~3Kb
~55Kb
E1'-E2'
E2'-E2
I1-E2
E2-I2
E1'-I1'
E1-E2
~9Kb
D.
E1'-E2'
E2'-E2
Relative Expression
Ctrl
E1 KD1
E1 KD2
UTR KD1
UTR KD2
E.
E1'-E2'
E2'-E2
Relative Expression
Ctrl
mbl-C KD1
mbl-C KD2
mbl-O/P KD1
mbl-O/P KD2
F.
rp49
tim
mbl-O/P
mbl-C
Relative Expression
Brain
Head
G.
mbl Distal Promoter Expression
mbl Proximal Promoter Expression
Lawf2
MBON-g1pedc
PB-1
MBON-bp1
Muscles-Head
PAM-9
R8-Rh6
PAM-8
PAM-2
PAM-7
R8-Rh5
PAM-5
PAM-1
R7-Rh3
Dm11
R7
L1
R1.6
mbl-O/P Expression
R7
R8-Rh5
R7-Rh3
R8-Rh6
R1.6
Pdf
Kdm2
Glia-Eg
PAM-5
MBON-bp1
PAM-1
Dm12
PAM-9
L1
PAM-2
Dm11
PAM-7
mbl-C Expression
Lawf2
PAM-1
Tm9
PB-1
PAM-9
MBON-g1pedc
mbl Distal Promoter

Figure S2.

Figure S2 related to Figure 1 and 2 : Mbl-C and O/P isoforms express in different cell types and use different promoters. A. qRT-PCR evaluation of *mbl-C* and *mbl-O/P* expression levels in Exon1 KD lines. **B.** Western blot analysis of Exon1 KD flies blotted against MBL antibody, tubulin was used as loading control. **C.** Scheme for the upstream promotor and conventional promoter *mbl* transcript qPCR primers. **D, E**. qRT-PCR evaluation of Relative expression levels of transcripts from Upstream and conventional promoter of *mbl* locus in *mbl* Exon1, UTR KD flies and *mbl-C, mbl-O/P* KD fly heads respectively. **F**. qRT-PCR evaluation of *rp49*, *tim* and *mbl* isoforms expression levels in fly brain and head. **G**. **Left panel**: mean *mbl* distal vs proximal promoter splice junction signal. **Central panel**: mean *mbl-O/P* vs *mbl* proximal promoter splice junction signal. **Right panel**: mean *mbl-C* vs *mbl* distal promoter splice junction signal. In all the qRT-PCR analysis tubulin was used as a normalization control (n = 3, error bars represent standard error of the mean, two-tailed t.test performed for significance difference (****, $p<0.0001$, ***, $p<0.0002$, **, $p<0.0021$, *, $p<0.0332$)).



Isoform	R7	R7-Rh3	R7-Rh3	R7-Rh3	R1
circMbl	16.3	15	17.6	16.4	8.5
circMbl2	11.9	23.28	6	13.3	6.4
circMbl3	17.6	16.4	10.5	15.4	11.4
circMbl4	35.6	28.7	28.3	27.4	2.6
circMbl5	10.4	13.1	3	11.6	2.7

Figure S3.

Figure S3 related to Figure 2: circMbl isoforms expression correlates with Mbl-C and Mbl-O/P isoforms expression in brain and photoreceptor cells. A. Example of reads supporting circMbl back-splice junction in 3'seq and 10X single cell data. **B.** Representation of pseudo chromosomes used for single cell annotation of each *mbl* isoform. **C.** Heatmap representation of mean *mbl* exon 2 normalized expression in each single cell cluster. **D.** Heatmap representation of mean circMbl normalized expression in each sorted cell cluster. **E.** Heatmap representation of mean *mbl-C* normalized expression in each sorted cell cluster. **F.** Mean normalized reads of each circMbl isoform in different photoreceptor neurons TAPIN-seq total-RNA sequencing. **G**. Mean circMbl4 vs *mbl-O/P* splice junction signal by cell-type in TAPIN-seq total-RNA sequencing (left panel), Mean circMbl4 vs *mbl-C* splice junction signal by cell-type in TAPIN-seq total-RNA sequencing (right panel).

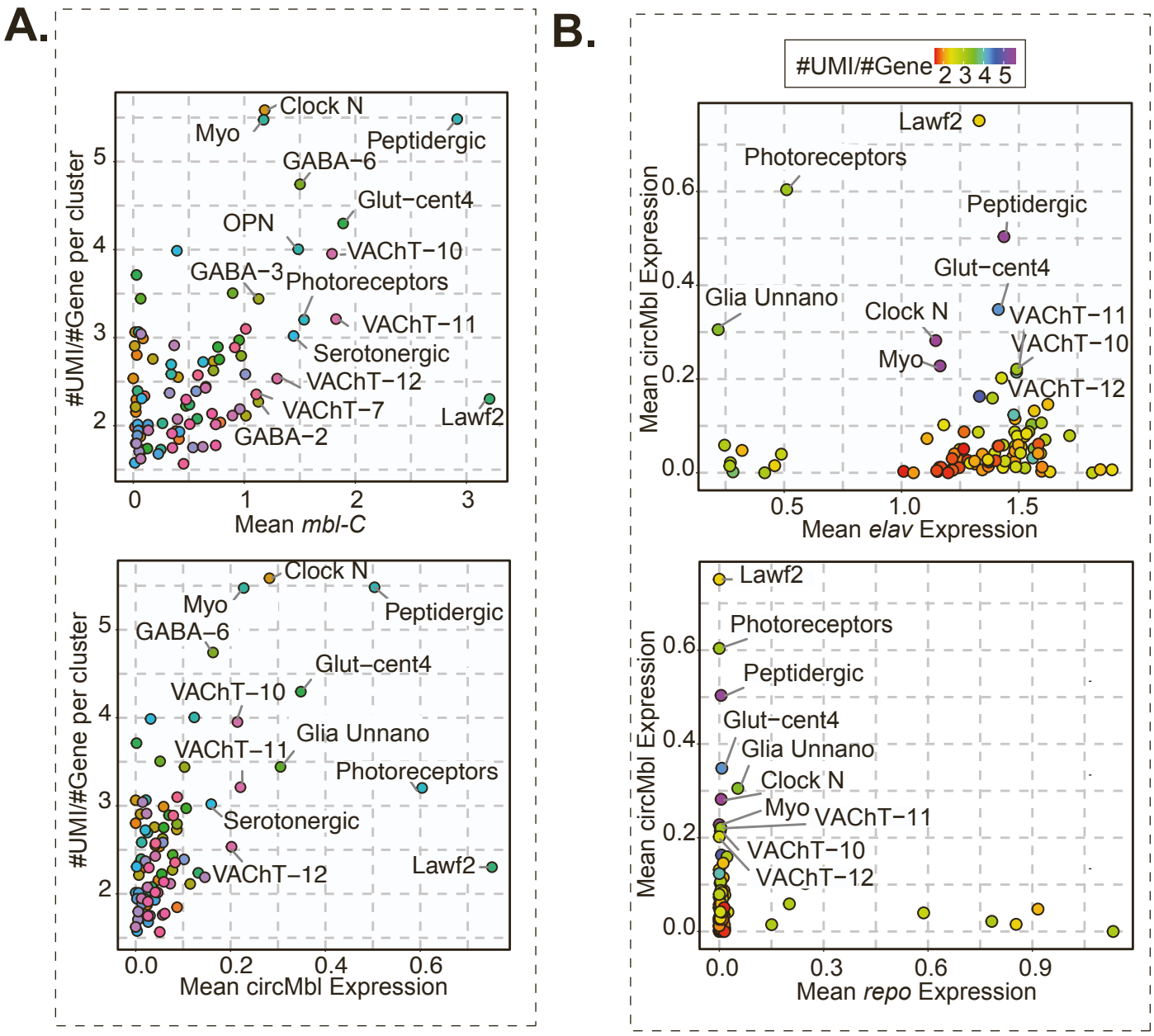

A.
B.
#UMI/#Gene
2 3 4 5
#UMI/#Gene per cluster
Mean mbl-C
Clock N
Myo
Peptidergic
GABA−6
Glut−cent4
OPN
VAChT−10
GABA−3
Photoreceptors
VAChT−11
Serotonergic
VAChT−12
VAChT−7
Lawf2
GABA−2
Mean circMbl Expression
Glia Unnano
VAChT−10
Mean elav Expression
Mean repo Expression

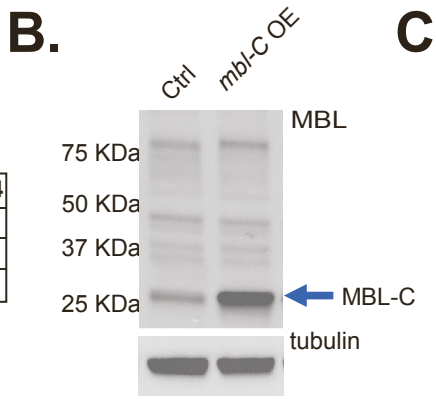
Figure S4.

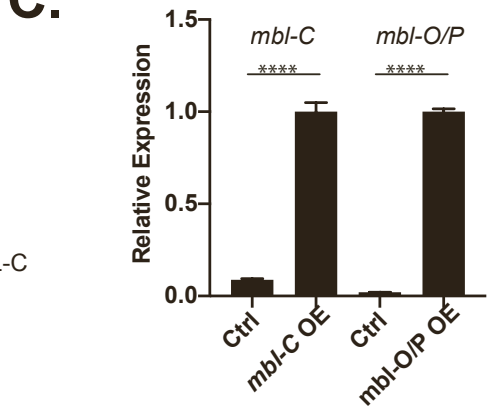
Figure S4 related to Figure 2: Analysis of circMbl in single cells data. A. Upper panel: mean normalized *mbl-C* reads vs the ratio of UMIs/Genes detected in each single cell cluster. **Lower panel:** mean normalized circMbl reads vs the ratio of UMIs/Genes detected in each single cell cluster. **B** Mean normalized circMbl reads vs mean *elav* and *repo* reads (upper panel and lower panel respectively). Color represents the ration of UMIs/Genes detected in each single cell cluster.

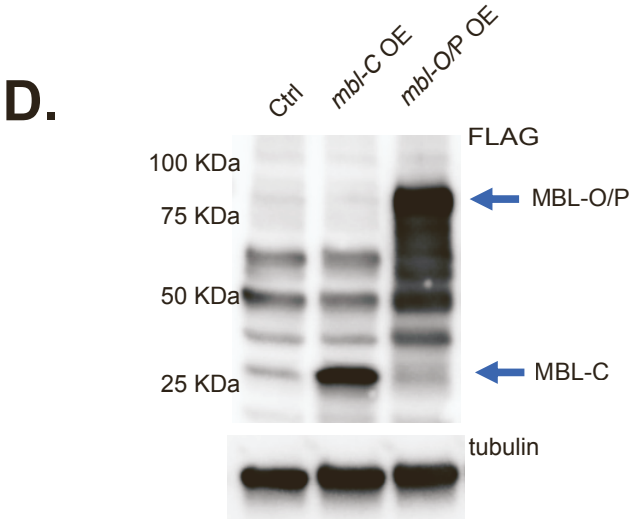
A.

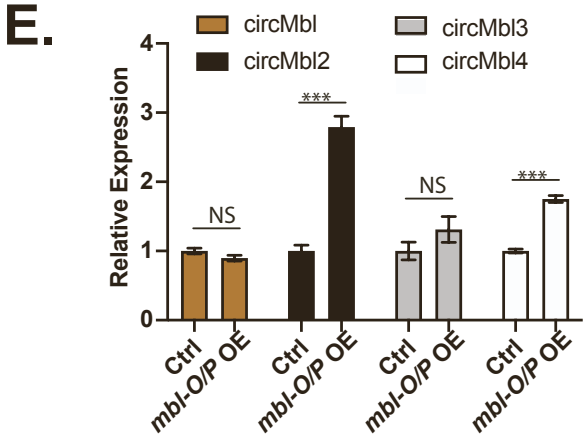
Developmental Viability

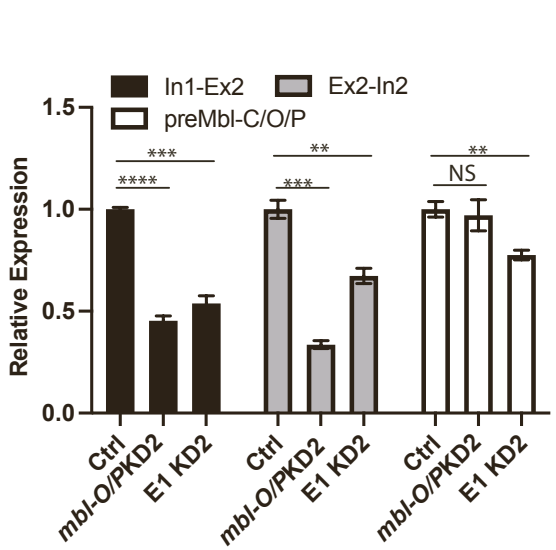
Fly Strains	Actin-Gal4	Elav-Gal4	Gmr-Gal4
mbl-C OE	0%	44 %	ND
mbl-C-FLAG OE	0%	ND	84 %
mbl-O/P-FLAG OE	0%	ND	100 %

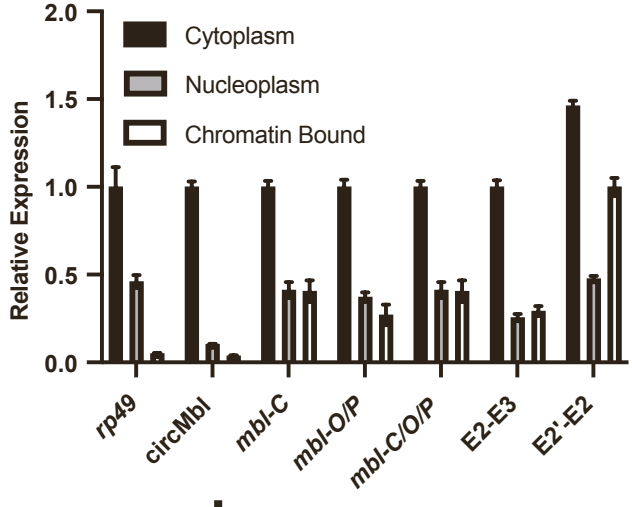


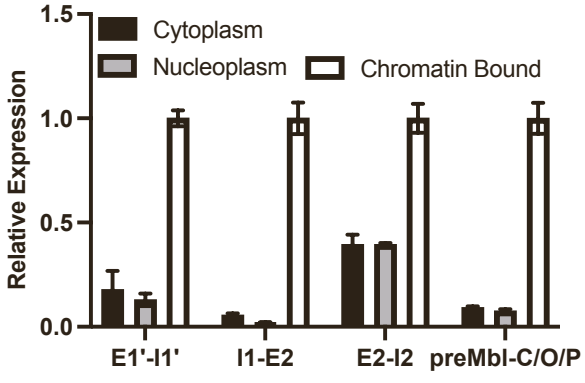


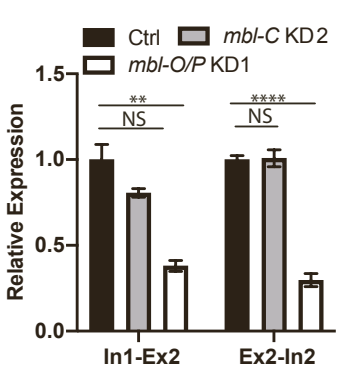












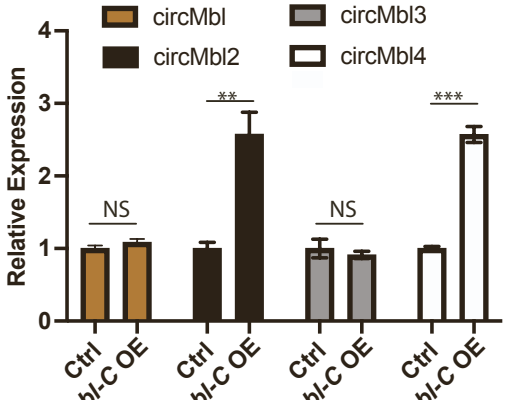
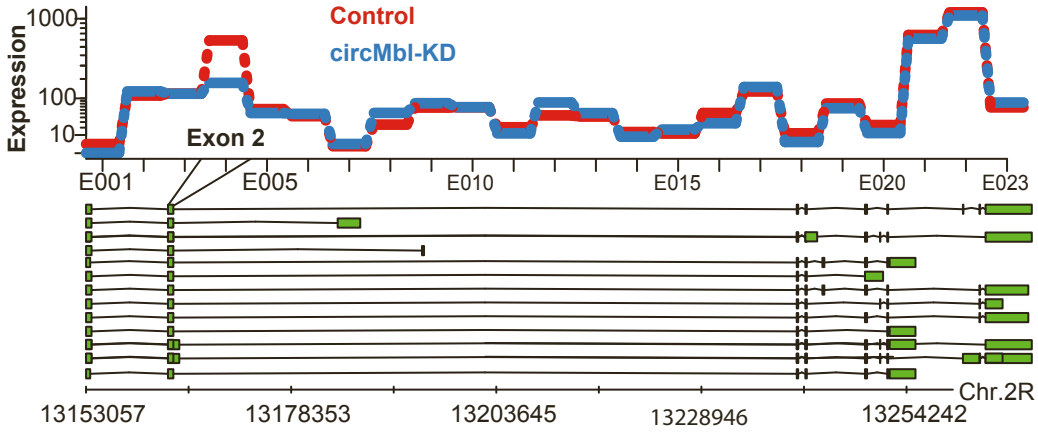


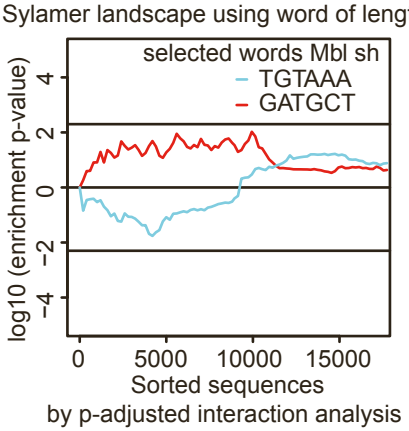
Figure S5.

Figure S5 related to Figure 3 and 4: MBL isoforms regulates its own expression by producing different circMbl isoforms in different cell types. A. Viability count for *mbl-C* and *O/P* isoform OE flies with different gal4 driver lines % was plotted against control sibling flies. **B.** Wester blot image showing levels of Mbl-C in *mbl-C* OE fly heads, membrane was blotted using anti-MBL, tubulin is blotted as a loading control. **C.** qRT-PCR evaluation of the levels of *mbl-C* and *mbl-O/P* isoforms in FLAG tagged *mbl*-OE fly heads. **D.** Western blot image showing levels of MBL-C and MBL-O/P in FLAG tagged *mbl-C* and O/P OE lines fly heads, membrane was blotted using anti-FLAG, tubulin is blotted as a loading control. **E.** qRT-PCR evaluation of the levels of circMbl isoforms in *mbl-O/P* OE fly heads. **F.** qRT-PCR evaluation of the levels of pre-RNAs in *mbl-O/P*, and Exon1 KD fly heads. **G, and H.** qRT-PCR evaluation of chromatin bound, Nucleoplasm, and cytoplasm fractions RNA of *rp49* and *mbl* locus isoforms from control fly heads. **I.** qRT-PCR evaluation of chromatin bound In1-Ex2, Ex2-In2 levels in control, *mbl-C,* and *mbl-O/P* KD fly heads. **J.** qRT-PCR evaluation of the levels of circMbl isoforms in *mbl-C* OE fly heads. In all the qRT-PCR analysis tubulin was used as a normalization control (n = 3, error bars represent standard error of the mean, two-tailed t.test performed for significance difference (****, $p<0.0001$, ***, $p<0.0002$, **, $p<0.0021$, *, $p<0.0332$)).

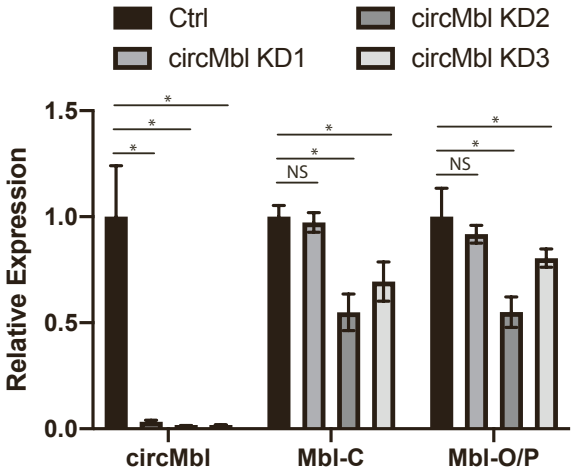
A.



B.



C.



D.

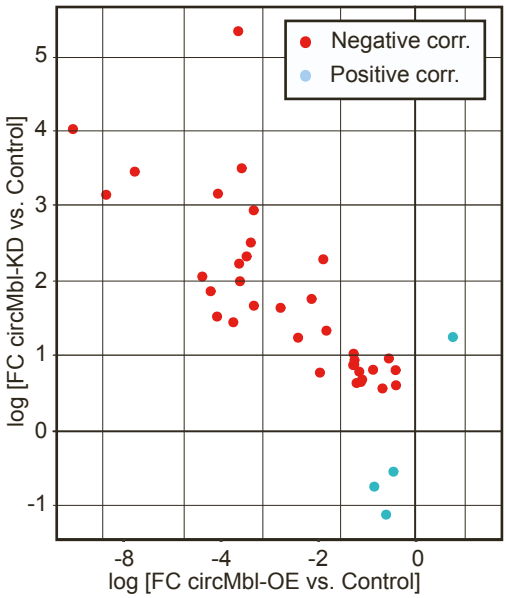


Figure S6.

Figure S6 related to Figure 5 and 6: circMbl can be knockdown specifically using shRNAs targeting the circMbl junction. A. The shRNA against circMbl does not affect alternative splicing/ other *mbl* isoforms expression. Data presented is differential exon usage analysis performed using DEXseq. **B.** Sylamer enrichment landscape plot for sh-circMbl and sh-circMbl* 6mers. The x-axis represents the genes sorted from the most to the least enriched in the AGO-1 IP-seq. The y-axis shows the hypergeometric significance for each word at each leading bin. **C.** qRT-PCR evaluation of circMbl, *mbl-C* and *mbl-O/P* expression levels in circMbl KD lines. **D.** Fold changes (in log scales) between circMbl OE (x-axis) and circMbl-KD (y-axis) in comparison with their respective controls. Blue indicates genes showing positive correlation in both comparisons. In red, the genes showing negative correlation between the fold change in one comparison and the other. In all cases we considered genes with fold change > 1.5 and p-value<0.05. In all the qRT-PCR analysis tubulin was used as a normalization control (n = 3, error bars represent standard error of the mean, two-tailed t.test performed for significance difference (****, $p<0.0001$, ***, $p<0.0002$, **, $p<0.0021$, *, $p<0.0332$)).

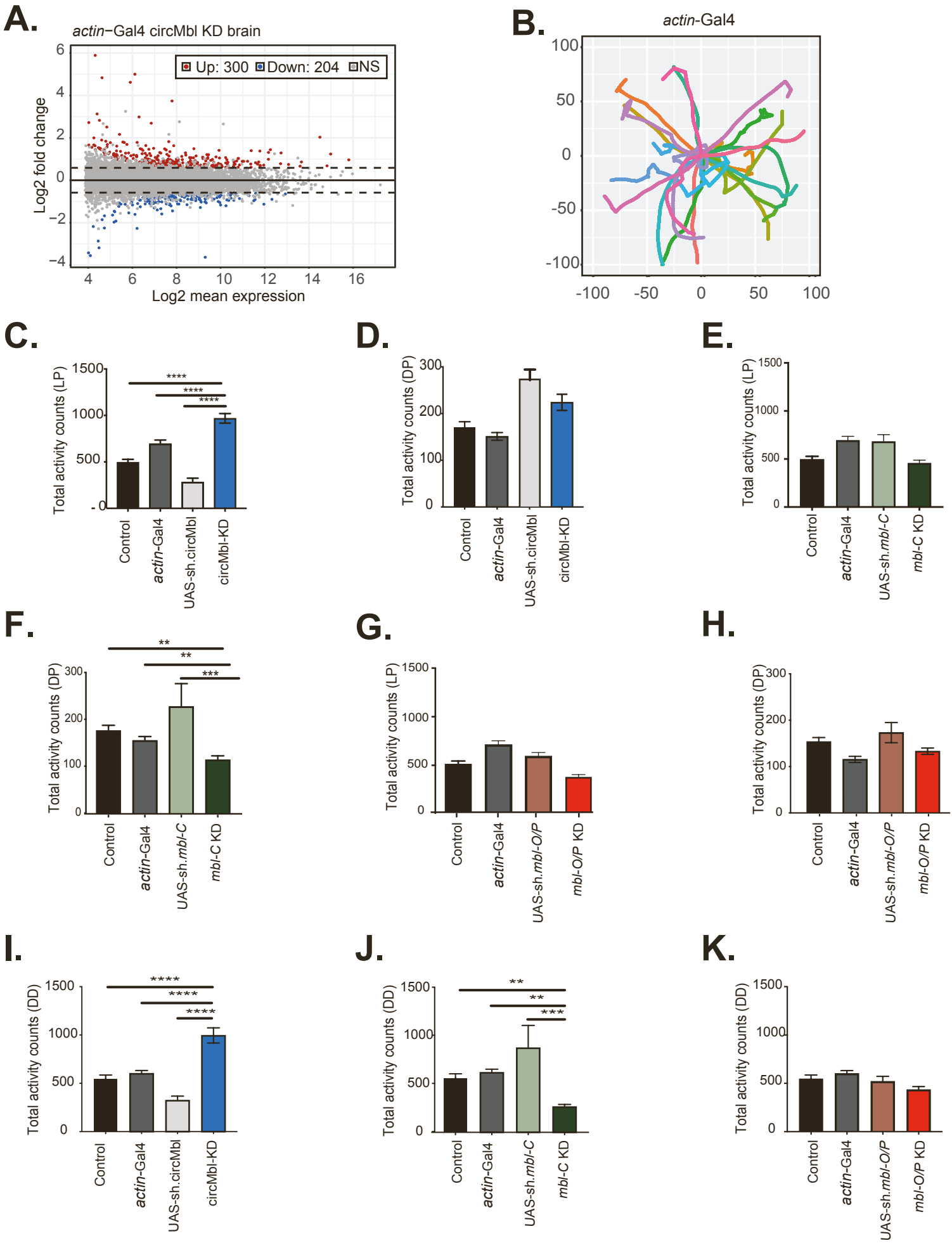

A.
actin−Gal4 circMbl KD brain
Up: 300
Down: 204
NS
Log2 fold change
Log2 mean expression
B.
actin-Gal4
C.
Total activity counts (LP)
Control
actin-Gal4
UAS-sh.circMbl
circMbl-KD
D.
Total activity counts (DP)
Control
actin-Gal4
UAS-sh.circMbl
circMbl-KD
E.
Total activity counts (LP)
Control
actin-Gal4
UAS-sh.mbl-C
mbl-C KD
F.
Total activity counts (DP)
Control
actin-Gal4
UAS-sh.mbl-C
mbl-C KD
G.
Total activity counts (LP)
Control
actin-Gal4
UAS-sh.mbl-O/P
mbl-O/P KD
H.
Total activity counts (DP)
Control
actin-Gal4
UAS-sh.mbl-O/P
mbl-O/P KD
I.
Total activity counts (DD)
Control
actin-Gal4
UAS-sh.circMbl
circMbl-KD
J.
Total activity counts (DD)
Control
actin-Gal4
UAS-sh.mbl-C
mbl-C KD
K.
Total activity counts (DD)
Control
actin-Gal4
UAS-sh.mbl-O/P
mbl-O/P KD

Figure S7

Figure S7 related to Figure 7: circMbl KD leads to change in specific set of genes and behavior.

A. MA plot showing gene expression differences in fly brains between control and *actin*-Gal4:circMbl KD flies. **B.** Graphical representation of path covered by 3rd instar *actin* Gal4 control larvae in larval assay. Each larvae is represented in a different color and X and Y axis indicate distance covered in mm. **C-F.** Quantification of total activity for ubiquitous knockdown of circMbl (n=120), and *mbl-C* (n=115) with corresponding 8MM, Gal4, and UAS controls, during the light period (LP) and dark period (DP) in 12:12 LD. **G-H.** Quantification of total activity for ubiquitous knockdown *mbl-O/P* (n=96) with corresponding 8MM, Gal4, and UAS controls, during the light period (LP) and dark period (DP) in 12:12 LD. **I-K.** Quantification of total activity over five days in complete darkness (DD). Stars represent statistical significance relative to 8MM and *actin*-Gal4 controls calculated by one-way ANOVA using Tukey's multiple comparisons test (****, $p<0.0001$, **, $p<0.005$)