Supporting Information

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SI Materials and Methods

Fly Lines. Myb interacting protein (*Mip*) 130, *Mip130*$^{1\text{-}36}$ (MMB core component), *mip130*$^{1\text{-}723}$, *mip130*$^{1\text{-}723}$; *myb*^{mh107}, *mip120*^{67}, *mip120*$^{67\text{-}21\text{-}6}$ were previously developed in our laboratory (1, 2). *L(3)mbt*^{gm76} (*lethal 3 malignant brain tumor*)/*tm3* (3) was a kind gift from the Lehman laboratory (Skirball Institute, New York). *Df(2L)e2f2*^{329} (transcription repressor)/*Df(2L)e2f2*^{329}; P[*e2f2*−; *mpp6*$^{+}$]/+/Cyo and *e2f2*$^{76Q.1}$/Cyo (4) were kind gifts from the Lipsick and Duronio laboratories (Stanford University School of Medicine, Stanford, CA and University of North Carolina, Chapel Hill, NC, respectively). *Gfp::piwi* (transposon regulator)/*cyo* line (5) was a kind gift from the Hannon and Aravin laboratories (Cold Spring Harbor Laboratory, New York and California Institute of Technology, Pasadena, CA, respectively). *Cyo/sco*, *tm3/cxd*, and *tm3 actin::gfp* were obtained from our fly stock.

Microscopy. Six to ten third instar larvae were dissected at room temperature on 1× PBS, and their salivary glands or brains were extracted by using fine tip dissection forceps. After removal of excess fat, the glands and brains were placed on a precleaned glass slide with 12 μL of PBS with 1:1,000 dilution of Dapi (4′, 6-diamidino-2-phenylindole) and covered with a 24 × 24 mm precleaned coverslip. Larval brains and some salivary glands were then sealed with rubber cement and directly imaged (unsquashed samples). Some salivary glands (squashed samples) were further processed as follows: the excess liquid was carefully removed with Kim wipes and the slides were repeatedly tapped lightly with the rubber end of a pencil. The slides were then sealed using rubber cement and then expeditiously imaged fresh. When differentiating YFP from GFP fluorophores (Fig. 2*A* and Fig. S5), a Leica TCS SP2 AOBS confocal microscope was used to acquire stacks of images that were deconvoluted using the commercial software Huygens Professional. The remaining samples were imaged using a Zeiss AxioObserverZ1_RSS compound fluorescent microscope. FISH and IHC samples were imaged using a Zeiss LSM710 confocal RSS microscope.

Immunohistochemistry and FISH. The protocols used for all IHC staining and FISH were obtained from previously published work (6, 7). The following modifications were introduced to the protocols: Once fixed, salivary glands were squashed by placing a no. 1 24 × 24 mm cover glass over the slides, inverting them over paper towels, and pressing down vigorously with two thumbs on the glass. All FISH probes were generated by cloning the 5′ UTR plus the N terminus of the desired genes into a commercial plasmid (Invitrogen Topo-TA). Sequenced plasmids were then amplified and the desired regions were restriction digested and gel extracted. DNA was quantified and then labeled using a DIG-Nick Translation mix containing digoxigenin-11-dUTP (Roche; 11745816910) according to the manufacturer's protocol. The four probes used were pooled into the following hybridyzation mix: 25% (vol/vol) pooled probe, 4× SSC, 1× Denhardt's, 0.4 mg/mL sonicated salmon sperm, 50% (vol/vol) formamide. The mix was boiled and stored at −20 °C until use, when it was reboiled and added directly to the slides. After FISH hybridization and before IHC slides were immunolabeled, samples were incubated for 1 h at room temperature in 1× blocking buffer [2.5% (wt/vol) nonfat milk, 0.5% BSA, 0.8% Nonidet P-40, 0.1% Tween 20, 1× Tris S (12 mM Trizma Base, 180 mM NaCl, pH 7.2)]. Slides were then incubated overnight at 4 °C in blocking buffer containing primary antibodies (1:20–1:150 dilutions) or serum from L(3)mbt immunized rabbit (1:50 dilution).

The following antibodies were used: affinity purified rabbit anti-Mip40, anti-Mip120, anti-Mip130, anti-Caf1/p55, and anti Myb (8), monoclonal anti-Di-methyl H4K20 (1:5 for IHC) anti-CycA (Santa Cruz Biotechnologies; 1:2,000 dilution for Western blot), serum from rabbits immunized with a GST::L(3)mbt N terminus fusion protein (1:50 for IHC and FISH), and rabbit anti-*dm*Piwi which was a kind gift from the Hannon laboratory. The secondary antibodies used were: goat anti-rabbit::Horseradish peroxidase (Sigma-Aldrich 170-6515), Sheep anti-Digoxigenin (Roche 1333089), Alexa fluor goat anti-rabbit 488nm (Molecular probes A11008), Alexa fluor goat anti-rabbit 568 (Molecular probes A11036), Alexa fluor 488nm donkey anti-sheep (Molecular probes A11015), and Alexa fluor 555nm donkey anti-sheep (Molecular probes A21436).

Chromatin Immunoprecipitation. These experiments were performed on S2 cells (including the immunoprecipitation themselves and the qPCR readout) according to protocols described previously (9, 10). qPCR experiments were done in triplicate and normalized to Actin 5C as endogenous control, the mock ChIP (NS) was normalized to a value of 1. The sequence of the primer sets used is available upon request.

Salivary qPCR. Fifty salivary glands were dissected for each experiment and placed in RNAlater RNA stabilization buffer (Qiagen). Total RNA was then extracted according to the manufacturer's protocol, and 1 μg was reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen; 18080-093), with conditions set for a single reverse transcription reaction per mRNA. The cDNA thus generated was then chloroform extracted and resuspended in deionized H_2O. qPCR reactions were performed using commercial qPCR mix (Maxima SYBR Green/ROX qPCR Master mix) and Platinum Taq DNA polymerase (Invitrogen; 10966-018) on an Applied Biosystems 7300 Real-Time PCR cycler and the results were analyzed with Applied Biosystems software. All qPCR reactions were done in triplicate, and most experiments were done twice, the results presented representing an average. Actin 5C primers were used as endogenous control. The primer sequences used can be provided upon request.

GST Pull Downs. L(3)mbt was cloned into the pGEX-6P to achieve expression of GST::L(3)mbt on bacteria. The resulting plasmids were transformed into a BL-21 strain of bacteria induced with 1 mM isopropyl-beta-D-thiogalactopyranoside for 4 h at 30 °C. Purification was done according to standard laboratory protocol. The cells were centrifuged for 10 min at 8,000 × *g*. The pellets were resuspended in buffer A [500 mM Tris·HCl, pH 8.0, 100 mM KCl, 1 M NaCl, 0.05% Nonidet P-40, 1 mM EGTA pH 8.0, 1 mM EDTA pH 8.0, 0.5 mM DTT, 1 mM PMSF, and 1× protease inhibitor (Roche Complete)]. After freeze-thawing, the resuspended cells were sonicated on ice five times for 30 s each and then centrifuged at 20,000 × *g*. The supernatants were incubated for 120 min with glutathione Sepharose 4 fast flow (GE Healthcare/Amersham; 17-5132-01) previously equilibrated with buffer A. The beads were then washed five times with buffer A and stored until required in 40% (vol/vol) glycerol at −20 °C. The bound beads were equilibrated with IP buffer (25 mM Hepes, 150 mM KCl, 1 mM EDTA, 1 mM EGTA, 10% (vol/vol) glycerol, 1 mM DTT, 0.4 mM PMSF 1 mM NaMBS, 0.5% Nonidet P-40) and then incubated with in vitro translated MMB proteins for 120 min. These were generated by cloning the cDNAs of the different MMB components into a pCS2^{+} plasmid generating constructs that were then used as tem-

plates for a commercial rabbit reticulocite IVT kit, using the manufacturer's instructions (Promega; TNT Quick Coupled Transcription/Translation system L2080). The beads were washed five times with IP buffer (the salt concentration of the buffer was increased to 1 M KCl in certain cases as indicated above), boiled, and then developed by Western blot with the corresponding antibodies.

Data Analysis. A previously published dataset product of ChIP-Seq using anti-L(3)mbt antibody (11) was mapped against the *piwi* genomic locus using Bowtie 0.12.5 and the *Drosophila* genome version Dme_r5.32 (12). The reads obtained were analyzed and graphed using the integrated genome browser (IGB) (13) and the University of California Santa Cruz genome browser (14). The previously published peaks at an FDR of 0.5 (10) were compared with those obtained by ModEncode dataset (15) using bedtools (16). The published ChIP-chip smoothed data (10) were used to call peaks with a log2 ratio (of ppt vs. input) greater that 0.5. These peaks were compared with the Richter dataset (Peaks FDR 0.5), subtracting those regions not present in the ChIP-chip map.

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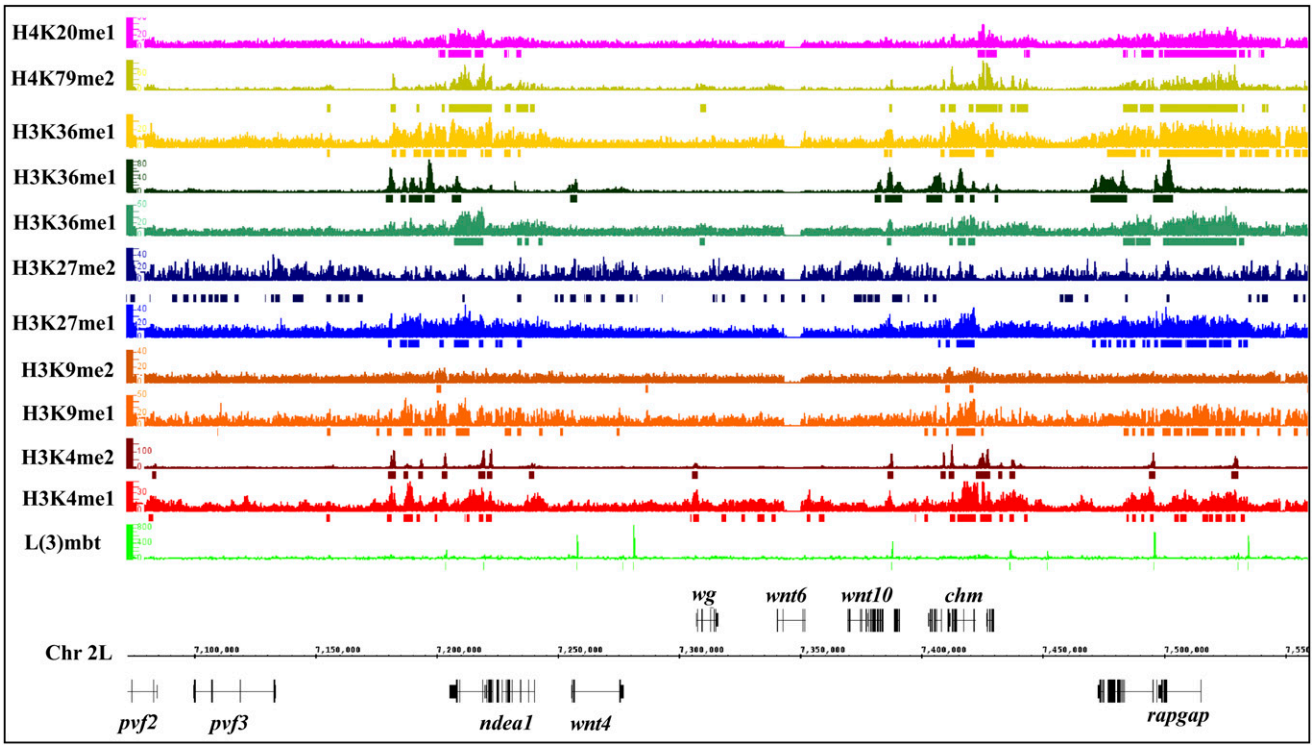

Fig. S1. Genome-wide comparison of the cytolocalization of lethal 3 malignant brain tumor [L(3)mbt] and mono- and dimethylated histones. Graphical representation of the Richter L(3)mbt brain ChIP-Seq (green) (1) and a subset of the modENCODE mono- and dimethylated histone Chip-Seq dataset tracks (2) (color coded as labeled, according to the Venn diagrams in Fig. 1*A* on the *wint* complex locus on chromosome 2L. The identity of the tracks are listed on the *Left*. Peaks are depicted as color coded bands below the corresponding tracks.

1. Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10(3):R25.
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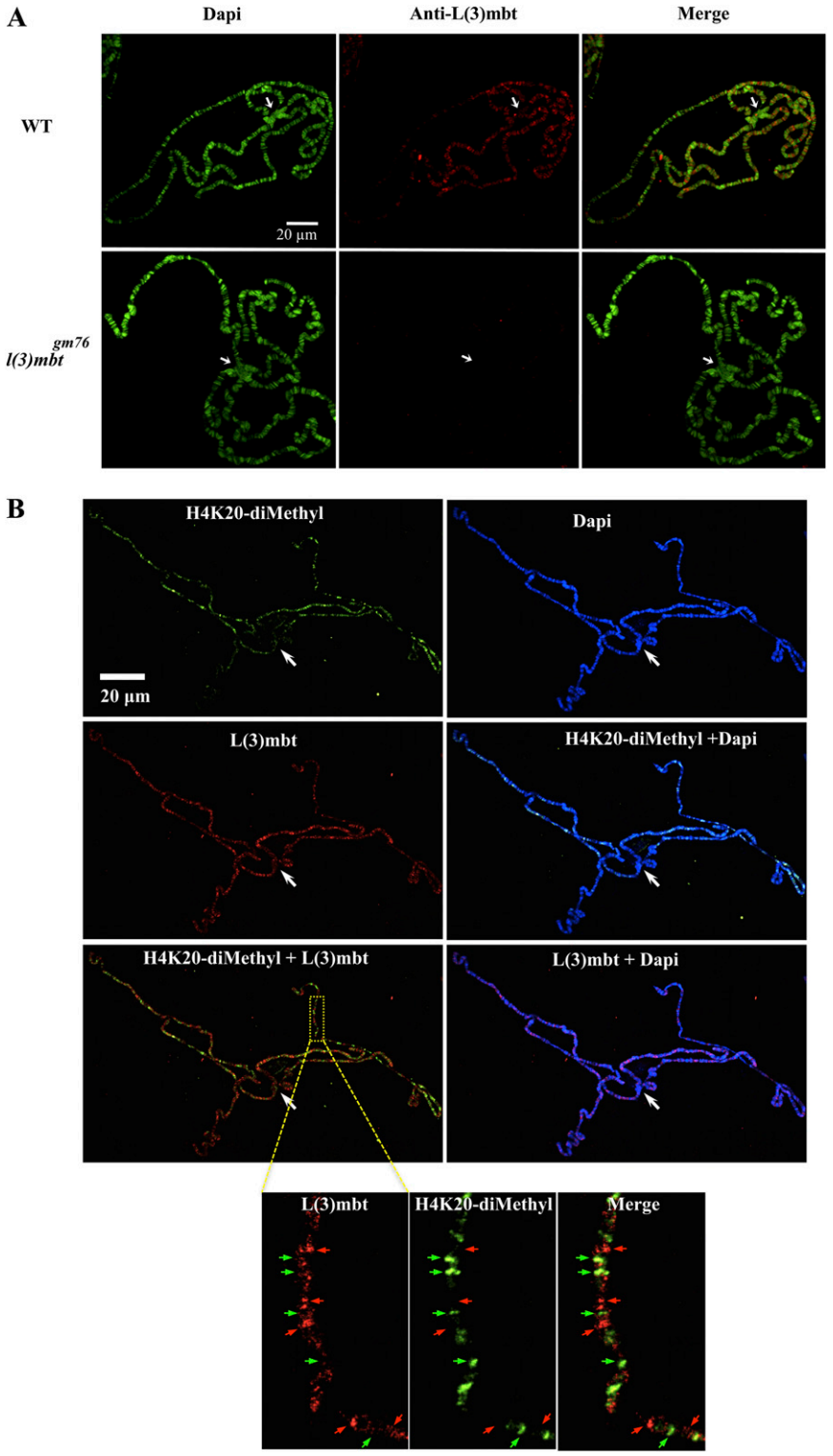

Fig. S2. Cytolocalization of L(3)mbt and H4K20 dimethyl on *Drosophila* polytene chromosomes by immunohistochemistry (IHC). (*A*) Immunohistochemical staining of wild-type and *l(3)mbt* putative null mutant polytene chromosomes. Polytene chromosome spreads from wild type (*Upper*) or *l(3)mbt*^{gm76} putative defective mutants (*Lower*) third instar larvae were stained with Dapi (shown in green) and then incubated with serum from rabbits immunized with the first (N-terminal) 441 amino acids of L(3)mbt fused to GST, and then stained with anti-rabbit fluorescently labeled secondary antibody (shown in red). In all panels, the white arrow indicates the centromeric heterochromatic. (*B*) Cytolocalization of L(3)mbt and H4K20 dimethyl on *Drosophila melanogaster* polytene chromosomes. A polytene chromosome spread from wild-type third instar larvae was stained with Dapi (in blue), rabbit serum anti-L(3)mbt (green), and monoclonal antibodies anti-H4K20 dimethyl (red). (*Top Left*) anti-L(3)mbt. (*Top Right*) Dapi. (*Middle Left*) Anti-H4K20 dimethyl. (*Middle Right*) Anti-L(3)mbt + Dapi. (*Bottom Left*) Anti-H4K20 dimethyl + anti-L(3)mbt. (*Bottom Right*) Dapi + anti H4K20 dimethyl. In all panels, the white arrow points to the centromeric heterochromatin. *B, Inset* is an amplification of the region highlighted by a dotted rectangle (*Bottom Left*). Red arrows indicate H4K20 dimethyl only bands and green arrows indicate L(3)mbt only bands.

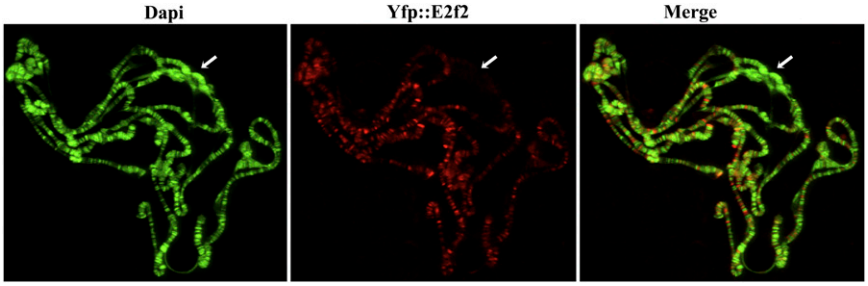

Fig. S3. Cytolocalization of YFP::E2f2 (repressor of transcription) on polytene chromosomes. Polytene chromosomes spread from *Drosophila melanogaster* third instar larvae carrying a *yfp::e2f2* transgene (red bands) were stained with Dapi (green bands) and imaged under a fluorescent compound microscope. Centromeric heterochromatin is indicated by a white arrow.

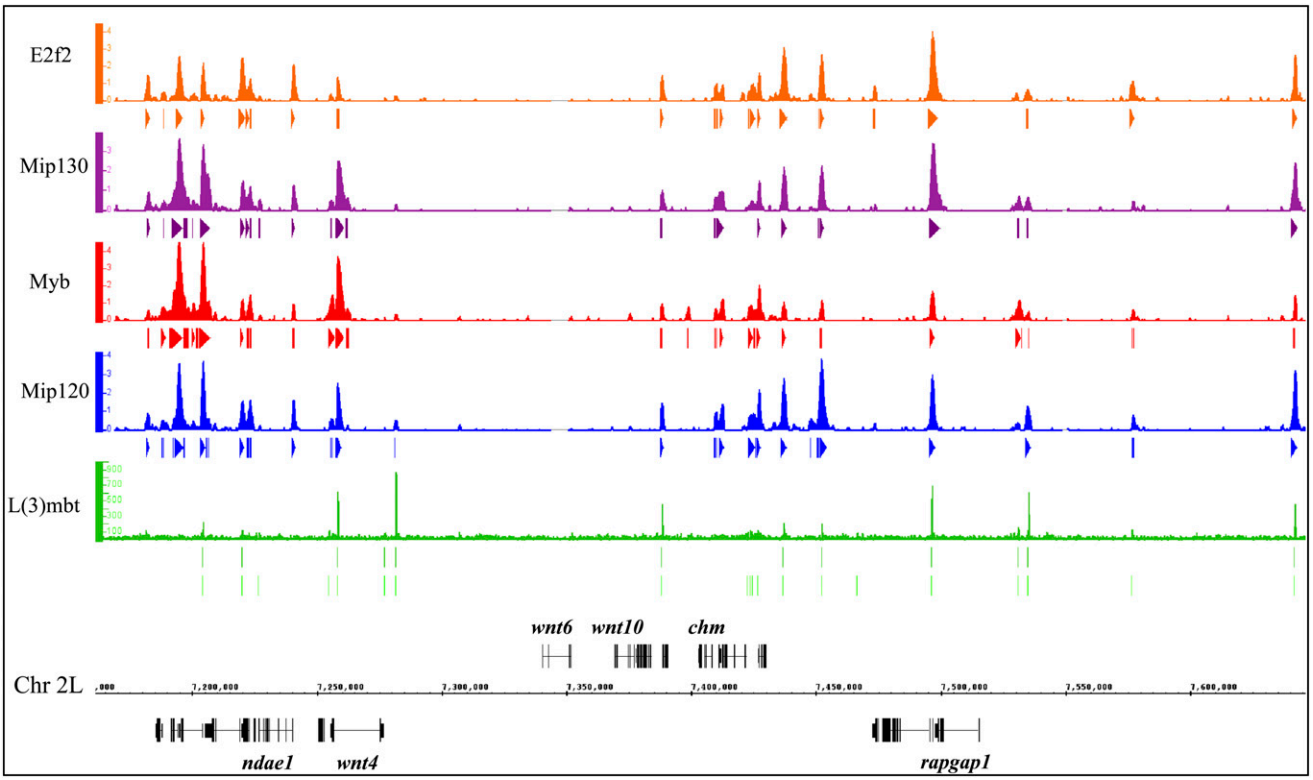

Fig. S4. L(3)mbt closely colocalizes with MMB complex members genome-wide. Graphical representation of the Richter L(3)mbt brain ChIP-Seq (green) (10) and the MMB KC cell Chip-chip dataset tracks (8) (color coded as labeled, according to the Venn diagrams in Fig. 2*F* on the *wnt* complex locus on chromosome 2L. Peaks are depicted as color-coded bands below the corresponding tracks. The identity of the tracks is on the *Left*.

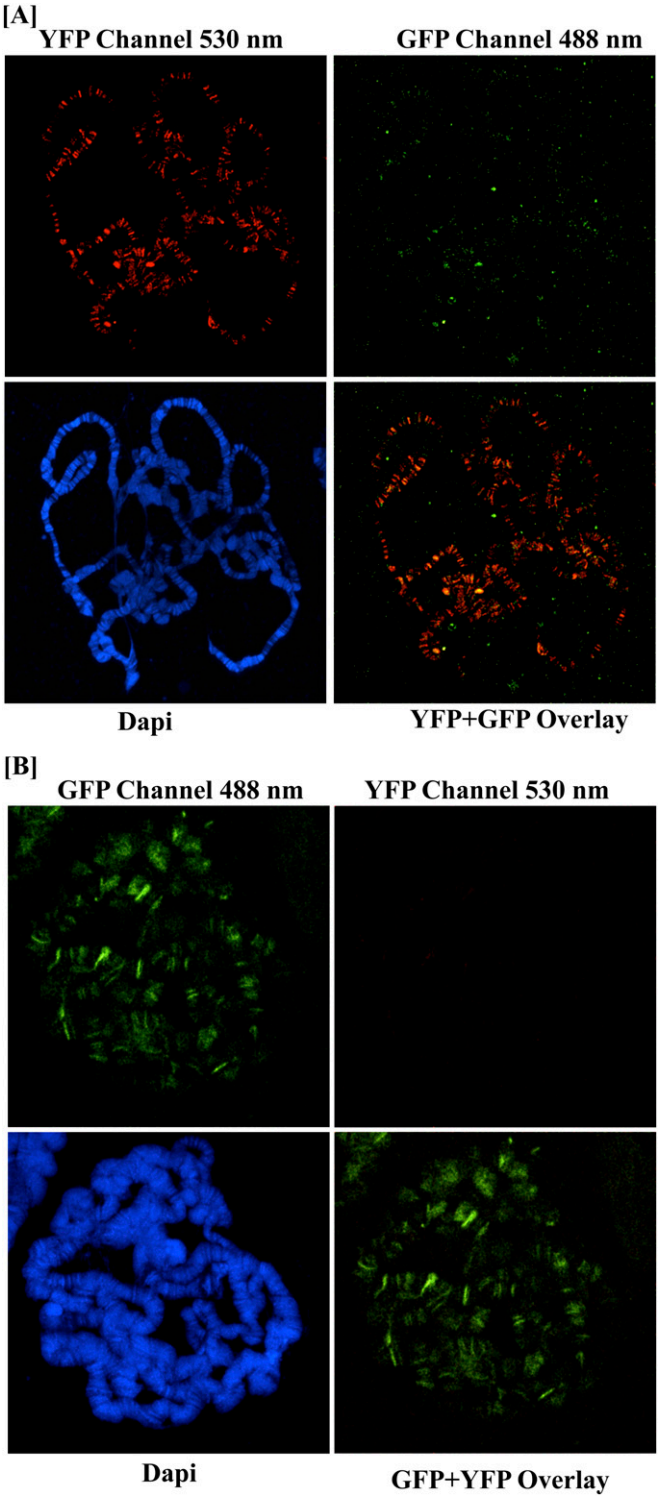

Fig. S5. Testing the wavelength overlap of YFP::E2f2 and GFP::Myb by confocal microscopy (*A*) Polytene Chromosome spreads from third instar larvae expressing YFP::E2f2 alone (red bands), stained with Dapi (blue bands), were tested for wavelength overlap in the GFP channel (*Right*, green bands). (*B*) Polytene Chromosome spreads from third instar larvae expressing GFP::Myb alone (green bands) stained with Dapi (blue bands), were tested for wavelength overlap in the YFP channel (*Right*, red bands).

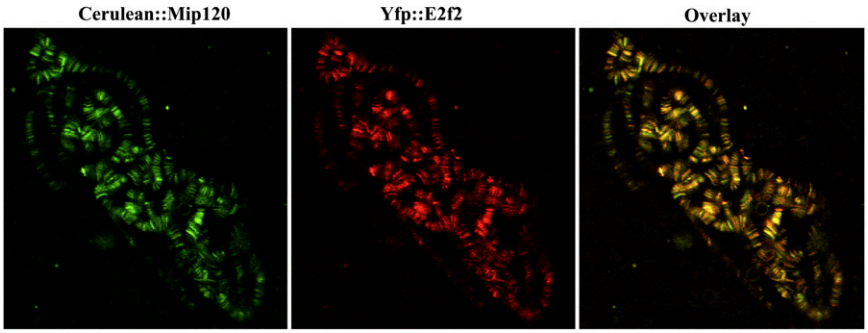

Fig. S6. Colocalization of YFP::E2f2 and Cerulean::Mip120. Polytene chromosome spreads from salivary glands of *Dm* third instar larvae coexpressing Cerulan::Mip120 (green bands) and YFP::E2f2 (red bands) imaged by fluorescent compound microscopy.

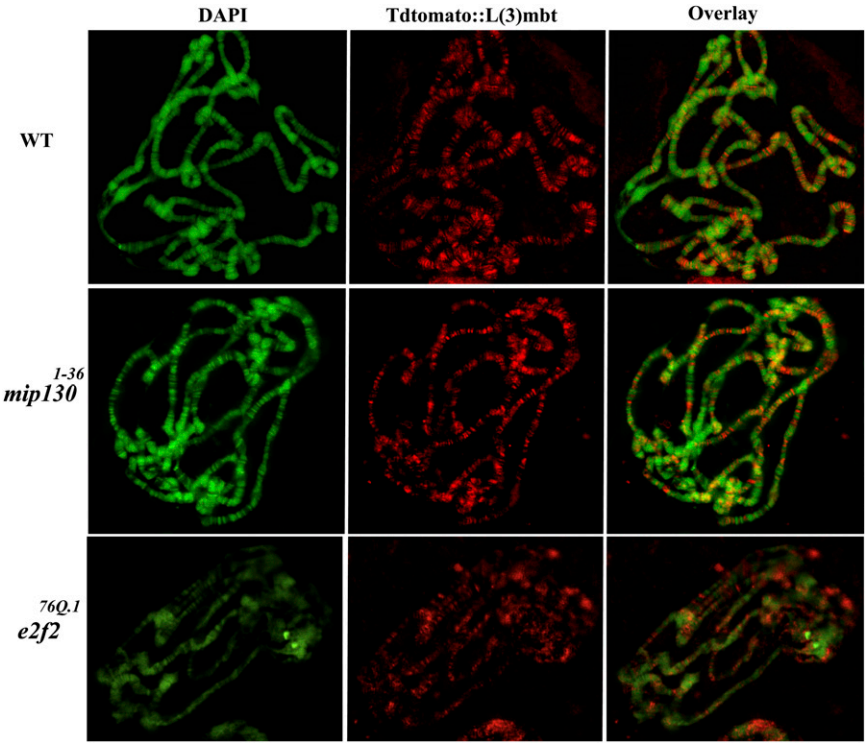

Fig. S7. Effect of deletion of MMB components on TDtomato::L(3)mbt. TDtomato::L(3)MBT (red bands) cytolocalization on polytene chromosomes stained with Dapi (green bands) from larvae either WT (*Top row*) or homozygous mutant for the following MMB complex members: $mip130^{1\text{-}36}$ (null mutant, *Middle row*). and $e2f2^{76q.1}$ (null mutant, *Bottom row*).