Figure EV1. RBPJ and L3MBTL3 co-localize genomewide in MDA-MB-231 cells.
A Venn diagram showing the genomewide co-localization of RBPJ and L3MBTL3
B Snapshots showing the co-localization of RBPJ and L3MBTL3 at the HES1 and HEY2 loci.

Figure EV2. KDM1A interacts with L3MBTL3 and RBPJ.
A L3MBTL3 and KDM1A interact in yeast two-hybrid assay. In this Y2H experiment, KDM1A is fused to the GAL4 DNA-binding (DB) domain and L3MBTL3 is fused to the GAL4 activation domain (AD). The DB-KDM1A and AD-L3MBTL3 fusion proteins interact with each other, leading to the activation of the HIS3 reporter gene and allowing yeast cells to grow on selective media lacking histidine. The six Y2H controls have been previously described (Dreze et al., 2010). The experiment was independently replicated thrice.
B Endogenous RBPJ interacts with both endogenous KDM1A and endogenous L3MBTL3. IP of RBPJ in U87-MG or MDA-MB-231 cells using a RBPJ antibody followed by Western blot analyses using KDM1A, L3MBTL3, or RBPJ antibody. The experiment was independently replicated twice.
C L3MBTL3 interacts with KDM1A in IP experiments. L3MBTL3 KO U87-MG cells were transfected with CRISPR/Cas9 sg-L3MBTL3-resistant plasmids encoding V5-L3MBTL3 WT or V5-L3MBTL3-A1(1-64) mutant. IPs were performed using V5 antibody and the precipitates were analyzed via Western blotting using V5, KDM1A, or RBPJ antibody. The experiment was independently replicated twice.
D Endogenous KDM1A interacts with FLAG-HA-tagged RBPJ. IP of FLAG-HA-tagged RBPJ in U87-MG cells followed by Western blot analyses using RBPJ or KDM1A antibody. The experiment was independently replicated twice.
E Endogenous RBPJ interacts with FLAG-HA-tagged KDM1A. IP of FLAG-HA-tagged KDM1A in U87-MG cells followed by Western blotting analyses using FLAG or RBPJ antibody. The experiment was independently replicated twice.
F The SAM domain of L3MBTL3 is required for the L3MBTL3/KDM1A interaction. HEK293T cells were transfected with HA-tagged KDM1A and FLAG-tagged L3MBTL3 [WT or mutants, represented in Fig 2A]. Upon IP with HA antibody, proteins were analyzed via Western blotting using HA or L3MBTL3 antibody. The experiment was independently replicated twice.

Data information: WB, Western blot; IP, immuno-precipitation; EV, empty vector control.
Source data are available online for this figure.
Figure EV2.
Figure EV3. The L3MBTL3-(1-64) domain is conserved in dL(3)mbt.

A Summary of the analysis of the amino acid sequences of human L3MBTL3 and Drosophila dL(3)mbt using a hidden Markov model (HMM) profile alignment approach (Soding, 2005). L3MBTL3-(11-50) and dL(3)mbt-(658-698) regions are conserved \( (P = 6 \times 10^{-5}) \). The consensus sequences identified in the HMM profile–profile alignment analysis are series of tildes, and amino acid letters that represent the calculated order of most frequent residues found at each position in the multiple sequence alignment analyses for L3MBTL3 or dL(3)mbt and their homologs across species. An "uppercase letter" refers to a residue having high conservation in the profile. A "lowercase letter" refers to a residue having significant conservation in the profile. A ~ symbol refers to a position where no single residue stands out as being the most conserved. Between the alignments, the symbols indicate the overall value of aligning a pair of residues at a particular position: . indicates a score between -0.5 and +0.5; + indicates a score between +0.5 and +1.5; | indicates a score > +1.5; "empty space" indicates a gap in the alignment.

B Schematic representation of the human L3MBTL3 and the Drosophila dL(3)mbt proteins. The analysis of the amino acid sequences using the HMM profile alignment approach generated highly confident alignments for seven conserved domains: the CSL interaction motif (CIM), the MBT domains #1, #2, and #3, the SAM domain, and the ZnF domains #1 and #2. P values are shown for each pair of conserved domains.

Figure EV4. Gain of dL(3)mbt suppresses Notch-induced hyperplasia in the Drosophila eye imaginal disk.

Flies were grown at 25°C. Eye imaginal disks dissected from third-instar larvae of the indicated strains were labeled with EdU (red; to mark dividing cells) and subsequently stained with α-dNICD (green) and α-elav (blue; to mark differentiated cells) antibodies. E1-Gal4 is an eye-specific UAS driver.

A–D Disks dissected from E1-Gal4 control larvae present a normal morphology with a clear linear demarcation of EdU-positive dividing cells at the level of the morphogenetic furrow (red arrow in panel D), an indentation that demarcates the boundary between elav-positive developing photoreceptors located posteriorly and elav-negative undifferentiated cells located anteriorly.

E–H HA-dL(3)mbt overexpression alone has minimal effect on disk size or proliferation compared to E1-Gal4 control.

I–L Ectopic expression of dNICD results in enlarged and distorted eye disks. Yellow arrows mark regions of high UAS-dNICD expression.

M–P Gain of dL(3)mbt significantly suppresses the dNICD-induced hyperplasia. Yellow arrows mark regions of high UAS-dNICD expression.

Q–T To assess the potential effects associated with UAS titration, the number of UAS constructs was normalized with UAS-GFP so that every genotype contained two UAS constructs. Disks were labeled with EdU (red) and counterstained with DAPI (blue). Note that the additional UAS-GFP transgenes do not affect the EdU staining pattern or overall disk morphology (compare panels A, E, I, and M to panels Q, R, S, and T, respectively), demonstrating that UAS titration is not responsible for the UAS-HA-dL(3)mbt;UAS-NICD phenotype.

Data information: At least 10 disks for each genotype were analyzed. Representative images are shown. Scale bars: 50 μm.
Figure EV4.
Figure EV5. Functional interaction between lag-1/RBPJ and lin-61/L3MBTL3 during Caenorhabditis elegans embryonic development.

Proportion of dead embryos (n ≥ 700) of N2 (N2 refers to the WT strain) and lag-1(om13) mutant animals fed with or without lin-61(RNAi) bacteria. The progeny of six to eight animals grown at 25°C was scored for embryonic lethality. Shown are means ± s.d. of duplicate experiments. EV, empty vector control.