

Supplemental Figure Legends

Figure S1. Identification of *tis11* and *pan2* alleles.

(A) Over-expressing a dominant-negative form of Mastermind (Mam^{DN}) in immature INPs partially suppressed the supernumerary neuroblast phenotype in *numb* hypomorphic brains. Mam^{DN} antagonizes the activation of Notch target genes. Bar graph is represented as mean \pm standard deviation. P-value: *** <0.005.

(B) Identification of the *tis11* locus as a genetic modifier of the supernumerary neuroblast phenotype in *brat* hypomorphic brains.

(C) Expression of *pan2* mRNA in wild-type or *pan2* homozygous mutant animals. *pan2*^{f00130} (*pan2*^{-/-}) is a null allele.

(D) Graphic illustration of the functional domains in Pan2. Asterisks correspond to the molecular lesions in enzymatically inactive Pan2^{D1039N,1041Q} transgenic protein. UCH: ubiquitin C-terminal hydrolase.

Figure S2. Brat likely recruits Tis11 to the BRE-ARE motif in the 3'UTRs of self-renewal gene transcripts.

(A) The 3'UTRs of *dpn* and *klu* mRNA. High-affinity BREs: darker yellow. Lower-affinity BREs: lighter yellow. AREs: red.

(B) B-boxes are required for Brat to bind Tis11. This is the reciprocal IP-WB analysis of the lysate shown in Fig. 2B.

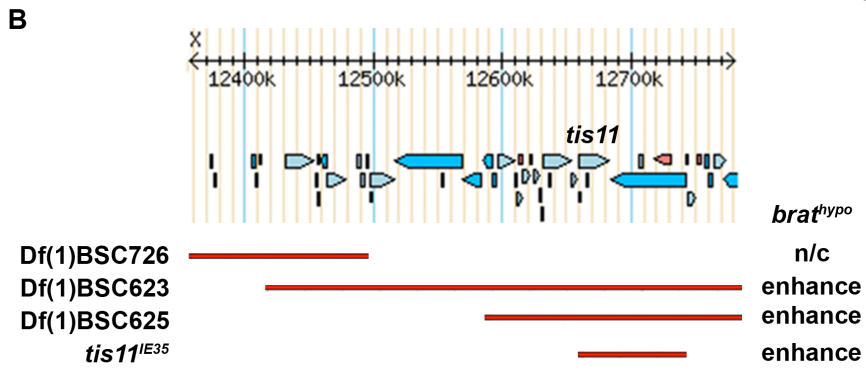
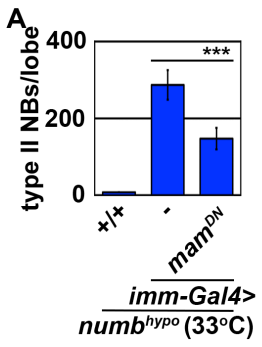
Figure S3. Conserved domains in Insb.

Illustration showing conserved motifs (marked in red) in Insb among 12 *Drosophila* species.

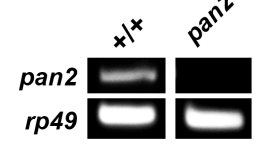
Figure S4. The Orange motif mediates Insb binding to Dpn.

C-terminally truncated Insb lacking the Orange motif fails to bind to Dpn. The Orange motif alone is sufficient to facilitate Insb binding to Dpn.

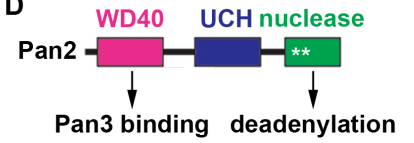
Komori_Supplementary_Figure 1



C



D



A

dpn 3'UTR

BRE: U/AUGUUAU/A

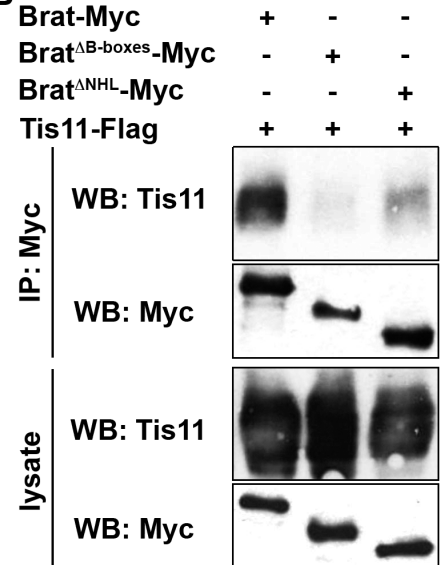
ARE: AUUUA

AUCAACGCA CAGGCCACUU CU**AUGUUA**CG UUUUAUC**AUU**
GUUUAUUUAU UUCAUUGCA**AUUUU**UUCAAA GAUCACUUCA
 AUGCUUUAGA ACCCAUUAU GUUUCAACGA UUUCUAU**AUG**
UUUUUAUGUA UCCCCGUUC GCUUUCUUC**AUUUU**AAGUUC
 AGCUGTG**AUU**U**GUUAU****AUUU**U**UUGUGU**AGU UCUCGUUUAA
 GCGCUUUUA CGACUAUUUA GUAGUUCAU AAAUAAUUUA
 GUUCUUUA**AU****GUUU**CAUUA CUGCUC**AAGU****AUUUA**AACAA
 UUAACGUGU UUCU**AUUUA**A GUUAUGUAGU UUUCAUUGUA
 UAGCGUUUA CAUAGGUGA UUCACGUUC UAUUUAGUG
 UUUUA**AUUUA**ACGAUUGUA**AUUUU**AACUUU UAGGUAAUGC
 AAAAUUCGAU GGAGUGAAAA UAAUGAUAAU AAAAAAAAAA
 UGCUGC

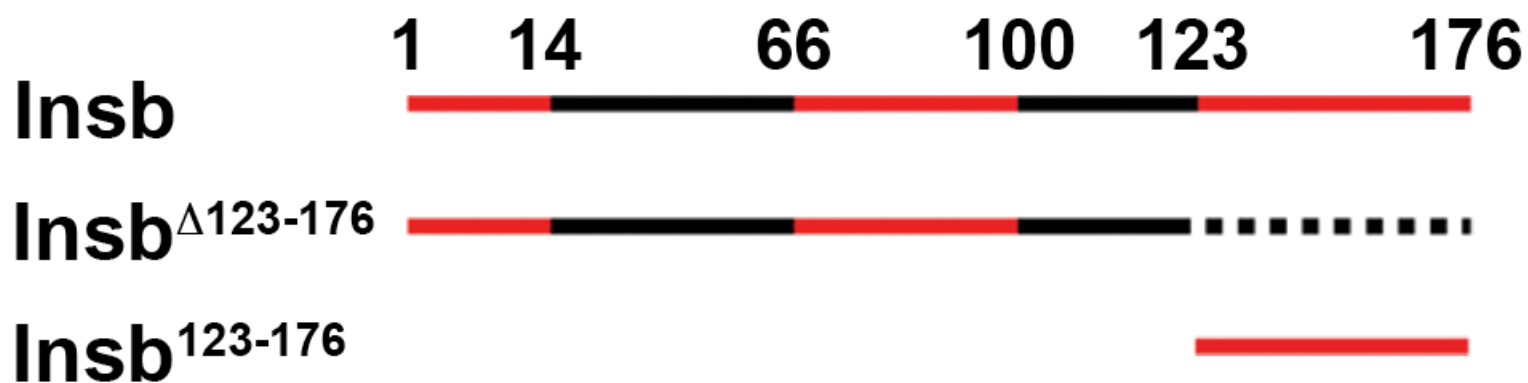
klu 3'UTR

CGGUGUCUGU AUGCAGCAGC AUCCAGACC**AUUUA**UGACCU GUUAGGUAAA
 AUCCAGUCCA UCCAUCUCC AUUUGACAGG CCCAGCUUAG UGCUACAGAA
 AAAUAUCUGA AAAAAAAAAUA GAGAAAAGAA AUGAAAACAU AUUAAACUAG
 AGCAGUUUGU ACUUGUACCG GAAUCUCUG CGAAGACUGA GGAACCUUAA
 UACUACCUAC AGUUUAAAUA AUAGGCUAAA GAACCAUCA CUAUUUUUGU
 AAAAGAGGAG UUAGAGGGCA GCACUCAUUA GACCACCCAA CGUAUUAGAA
 GUUUAACUAG UUCUAGUUGC UAGAUCUUU CGCGCGUCA AUGAACCCCC
 AGGAGUUUAG AUAGCCAGGA UCUUCGAUAC AGCUAGCAA UCCAUGUUC
 ACACCUAGGC UAUACUACAC UAUUAUAGA UAUUAUUA UAGUAUGGG
 UCAGAUCCA CGUCCCGAUG AUCUGAUCUC UCAGCAUUUU GAGCAACGUU
 GAAAUUACUA AGUAGACUC GCUACUUAGG CUUCAUAGGC ACGCCGCAUU
 GGAAAAGGCA UUACUUUUGU ACUCCUA**AUU**U**AAUUA**UU CUUA**AUUUA**U
 UUGUAACAU ACUCAGCAGA AUGUAAACUU AUACCACAAC GAUUUGUAUU
AUUUAAUUU UACCUU**AUU**AAU**AUUUA**U**UU**ACUUAGU UUUAAAGGGAU
 GCCUGCCUAC ACAAGAACAC AAACAAAAGA CAAAGAAUUC UCCCUAGGGC
 UCAAAAAGAA AAAAGAAACG AACGCUACAC AAAACUCAU UCUAUGGAAA
 UCUUAAAUCU UUAUUGGAAA AGAAAGACAA AAAGAAACAA UCCUUU**UUU**
UGUUCAACAA AAACACUGUU AA**AUUUA**CAU UACCUUUGUA AUUG**AUUUA**A
 UGCUUGCUCU UCUAUUAUCU AAACUACUUU **UUGUUUU**CAU GAAUUGAAAU
 AAAACUUUU AAGUUUUCUU UAGUUUAUA ACCAUCUGGG GAUUGGAAAC
 ACCUCACCC CUUCCGCUC CGCAAGUGGG GACACGUUA CCCGUUUCUG
 CCCCAGGGG UGGGAUCAC CUCACCUUUC GUGGUCGUUU AC**AUGUUUA**
 AGUUAAGUU GUUCGUU**AUU**UAUUAUAAA AGAAUUGAAA CAAAGCCCG
 ACUCCGAUU AUUGAUUAU AGUUCUAGC UAAACGUUA AAUAAUUUAU
 UA**AUUU****UGU**UAUUCUAGUU GGUUAUGAU AGCACUAGU UAAAAACGUU
 UUUCACAAA UAAAACAAA CAAAGUCUA ACAUGAUGGA CAAUGUCAUA
 CAAAACAGA**AUUUA**AAGCC

B



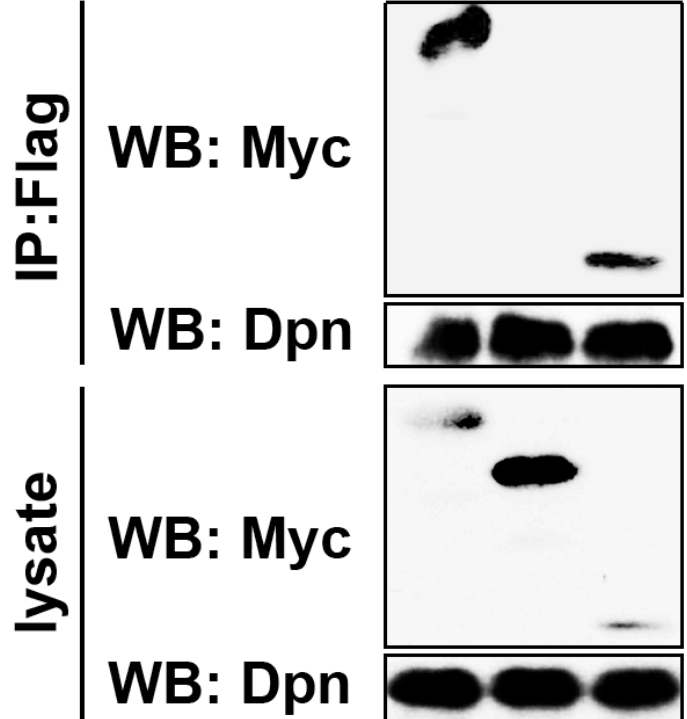
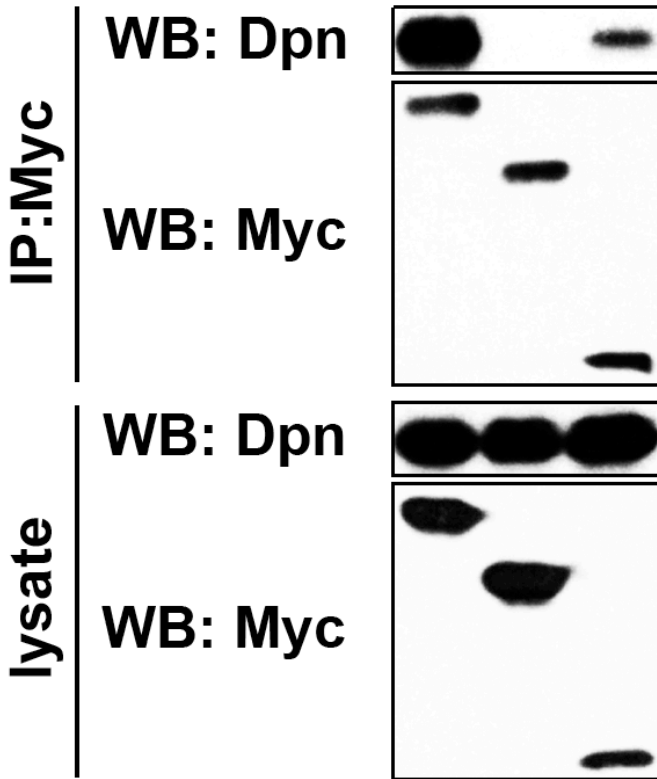
Komori_Supplementary_Figure 3



Komori_Supplementary_Figure 4

Insb::Myc	+	-	-
Insb^{Δ123-176}::Myc	-	+	-
Insb¹²³⁻¹⁷⁶::Myc	-	-	+
Dpn::Flag	+	+	+

Insb::Myc	+	-	-
Insb^{Δ123-176}::Myc	-	+	-
Insb¹²³⁻¹⁷⁶::Myc	-	-	+
Dpn::Flag	+	+	+



Komori Supplemental table 1

X		Name	Deleted segment	Sequence coordinates	Phenotypic effect	Responsible gene	Reference
BL stock #		Df(1)ED6565	2B14;2F5	X:1894112; 2317631	Suppressor	<i>arm</i>	Komori et al., <i>Development</i> 2014
		Df(1)BSC625	11B2;11D5	X:12583216;12974678	Enhancer	<i>CKI α, tss11</i>	Komori et al., <i>Development</i> 2014; this study
2nd		Symbol	Deleted segment	Sequence coordinates	Phenotypic effect	Responsible gene	Reference
BL stock #		Df(2L)ED105	21E2;22A1	2L:852854;1420528	Enhancer	unknown	This study
		Df(2L)ED94	21E2;21E3	2L:568095;1036969	Enhancer	unknown	This study
		Df(2L)BSC688	22B1;22D6	2L:1736964;2273384--2273572	Enhancer	<i>erm</i>	Jenssens et al., <i>Development</i> 2014
		Df(2L)BSC292	23F6;24A2	2L:3515462;3632008	Enhancer	unknown	This study
		Df(2L)ED690	30B3;30E4	2L:9437469;9918174	Enhancer	<i>numb</i>	This study
		Df(2R)Exel7094	44A4;44B5	2R:8061165; 8131743	Enhancer	unknown	This study
		Df(2R)ED1791	44F7;45F1	2R:8922730;9553252	Enhancer	<i>par2</i>	This study
		Df(2R)BSC408	45D4;45F4	2R:9292659;9578616	Enhancer	<i>not1</i>	This study
		Df(2R)BSC161	54B2;54B17	2R:17304783;17484828	Enhancer	<i>insb</i>	This study
		Df(2R)BSC355	54B16;54C3	2R:17462347;1753673	Enhancer	<i>insb</i>	This study
3rd		Symbol	Deleted segment	Sequence coordinates	Phenotypic effect	Responsible gene	Reference
BL stock #		Df(3L)ED4288	63A6;63B7	3L:3070827; 3149091	Enhancer	<i>par3</i>	This study
		Df(3L)BSC377	67E5;68A4	3L:10654713--10654743; 11070652	Suppressor	<i>klv</i>	Xiao et al., <i>Development</i> 2012
		Df(3L)BSC574	68F1;68F1		Enhancer	<i>pop2</i>	This study
		Df(3L)brm11	71F1-4;72D1-10		Enhancer	<i>brm</i>	Jenssens et al., <i>Development</i> 2014
		Df(3R)Exel7327	89A8;89B1 (DF)	3R:15901433--15901434; 16041562	Enhancer	<i>mor</i>	Jenssens et al., <i>Development</i> 2014
		Df(3R)DG2	89E1-1;4;91B1-B2		Enhancer	<i>osa</i>	Jenssens et al., <i>Development</i> 2014
		Df(3R)BSC56	94E1-2;94F1-2		Enhancer	<i>pppp1</i>	Komori et al., <i>Development</i> 2014
		Df(3R)Exel9012	94E9;94E13	3R:23279758; 23346387	Enhancer	<i>pnlp1</i>	Komori et al., <i>Development</i> 2014
		Df(3R)crib-F89-4	95D7-D11;95F15		Enhancer	<i>apc2</i>	Komori et al., <i>Development</i> 2014
		Df(3R)ITP	97A;98A1-2	3R:26059954--26078012; 27388608--27480200	Suppressor	unknown	This study
		Df(3R)3450	98I3;99A6-8		Suppressor	unknown	This study
		Df(3R)1127	99B5;6;99F1	3R:29468941--29565244; 30387804--30458521	Enhancer	<i>Akn</i>	Komori et al., <i>Development</i> 2014