Supplementary Materials for

Natural variation of the expression pattern of the segmentation gene *even-skipped* in *Drosophila melanogaster*

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Table S1. Significance tests for stripe height

position	time class	p value	lines compared
8	4	2.578690×10^{-8}	L1 L2
8	5	2.312320×10^{-8}	L1 L2
9	4	1.309975×10^{-5}	L1 L2
9	5	2.312320×10^{-8}	L1 L2
2	4	6.549873×10^{-6}	L1 L5
2	5	5.278690×10^{-4}	L1 L5
2	7	7.342234×10^{-5}	L1 L5
3	4	2.654761×10^{-5}	L1 L5
3	7	1.101335×10^{-4}	L1 L5
6	7	4.699030×10^{-4}	L1 L5
6	8	5.292265×10^{-5}	L1 L5
7	8	5.292265×10^{-5}	L1 L5
8	8	2.646133×10^{-5}	L1 L5
9	8	2.646133×10^{-5}	L1 L5
12	8	7.938398×10^{-4}	L1 L5
1	6	2.862549×10^{-4}	L2 L5
1	7	2.010140×10^{-4}	L2 L5
2	7	2.712094×10^{-5}	L2 L5
2	8	7.654884×10^{-4}	L2 L5
3	4	2.756877×10^{-4}	L2 L5
3	5	8.017171×10^{-4}	L2 L5
3	7	2.712094×10^{-5}	L2 L5
5	7	6.611727×10^{-4}	L2 L5
6	6	4.483716×10^{-4}	L2 L5
6	7	3.988374×10^{-7}	L2 L5
6	8	2.835142×10^{-6}	L2 L5
7	7	2.712094×10^{-5}	L2 L5
7	8	2.835142×10^{-6}	L2 L5
8	4	2.578690×10^{-8}	L2 L5
8	5	4.242361×10^{-9}	L2 L5
8	6	5.565384×10^{-4}	L2 L5
8	8	8.505426×10^{-5}	L2 L5
9	4	7.736070×10^{-7}	L2 L5
9	5	8.484722×10^{-9}	L2 L5
10	8	3.402171×10^{-5}	L2 L5
11	4	5.278578×10^{-5}	L2 L5
11	7	2.586460×10^{-4}	L2 L5
11	8	1.984600×10^{-5}	L2 L5
12	8	2.750088×10^{-4}	L2 L5

Table S1 The table shows all the significant pairwise rank sum tests for stripe height after Bonferroni correction. The positions are numbered 1 to 12. ‘1’ indicates the posterior half of stripe 1 height, ‘12’ indicates the anterior half of stripe 7 height. ‘2-11’ denote sequentially anterior and posterior of stripes 2-6 height. Time class is as indicated.

Table S2. Significance tests for normalized stripe height

position	time class	p value	lines compared
4	4	2.214837×10^{-4}	L1 L2
8	4	3.094428×10^{-7}	L1 L2
8	5	2.312320×10^{-8}	L1 L2
9	4	1.112189×10^{-4}	L1 L2
9	5	2.312320×10^{-8}	L1 L2
4	4	8.731444×10^{-5}	L2 L5
5	4	6.293035×10^{-4}	L2 L5
6	7	5.276619×10^{-4}	L2 L5
6	8	5.386770×10^{-5}	L2 L5
7	8	7.654884×10^{-4}	L2 L5
8	4	2.578690×10^{-8}	L2 L5
8	5	4.242361×10^{-9}	L2 L5
9	4	5.278578×10^{-5}	L2 L5
9	5	2.969653×10^{-8}	L2 L5

Table S2 The table shows all the significant pairwise rank sum tests for normalized stripe height after Bonferroni correction. See table S1 legend for details about position and time class.

Table S3. Significance tests for stripe width

position	time class	p value	lines compared
1	5	1.040544×10^{-6}	L1 L2
3	6	1.145039×10^{-3}	L1 L2
5	5	3.588720×10^{-5}	L1 L2
5	6	3.391413×10^{-4}	L1 L2
7	5	4.112780×10^{-4}	L1 L5
1	7	5.276619×10^{-4}	L2 L5
2	5	4.062358×10^{-4}	L2 L5
2	6	1.031404×10^{-3}	L2 L5
4	8	1.040497×10^{-3}	L2 L5
5	4	1.114922×10^{-3}	L2 L5
5	5	1.582401×10^{-6}	L2 L5
5	6	2.708177×10^{-5}	L2 L5
6	8	7.654884×10^{-4}	L2 L5

Table S3 The table shows all the significant pairwise rank sum tests for the stripe width after Bonferroni correction. The ‘position’ denotes the stripe number.

Table S4. Significance tests for interstripe width

position	time class	p value	lines comed
1	4	2.214837×10^{-4}	L1 L2
3	5	4.448903×10^{-4}	L1 L2
3	6	1.748151×10^{-6}	L1 L2
3	7	4.824896×10^{-5}	L1 L2
3	8	1.058453×10^{-4}	L1 L2
4	4	1.309975×10^{-5}	L1 L2
4	8	5.027652×10^{-4}	L1 L2
1	4	1.186674×10^{-3}	L1 L5
3	4	9.618514×10^{-6}	L2 L5
3	5	4.242361×10^{-9}	L2 L5
3	6	2.136629×10^{-7}	L2 L5
3	7	9.671807×10^{-6}	L2 L5
3	8	5.528527×10^{-4}	L2 L5
4	4	1.761245×10^{-5}	L2 L5
4	5	1.293801×10^{-3}	L2 L5
4	6	4.483716×10^{-4}	L2 L5
4	8	1.275814×10^{-4}	L2 L5
5	6	4.844806×10^{-5}	L2 L5
5	8	3.940848×10^{-4}	L2 L5

Table S4 The table shows all the significant pairwise rank sum tests for interstripe width after Bonferroni correction. The ‘position’ ranges from 1 to 6, where ‘1’ represents the 1-2 interstripe and so on.

Table S5. Gene list

gene name	chromosome	begin	end
bcd	chr3R	2579564	2587188
cad	chr2L	20768731	20785135
ftz	chr3R	2688046	2693966
gt	chrX	2319146	2325000
hairy	chr3L	8666859	8674353
hb	chr3R	4514702	4525544
kni	chr3L	20683430	20689656
Kr	chr2R	21112134	21120917
odd	chr2L	3602224	3608756
paired	chr2L	12080995	12087827
runt	chrX	20559697	20570348
slp1	chr2L	3823675	3829099
slp2	chr2L	3834840	3841185
tll	chr3R	26676037	26682122
eve	chr2R	5860288	5876667

Table S5 The table shows the genes and their genomic locations used to detect large deletions or insertions that are present in L2 but absent in L1 and L5. The sequences are from 2kb upstream and downstream of each protein coding gene (RefSeq track from UCSC browser on 9.18.2014), except *eve*, which used 16kb whole-locus sequence.

Table S6. Number of embryos collected for each line

	T4	T5	T6	T7	T8
L1_original	24	12	14	10	13
L1_OK_orientation	15	12	10	9	12
L1_final	15	12	10	9	8
L2_original	19	19	14	13	15
L2_OK_orientation	18	19	13	13	13
L2_final	14	18	13	13	11
L5_original	16	14	15	18	12
L5_OK_orientation	15	14	15	14	11
L5_final	15	14	15	14	11

Table S6 There are three numbers indicated for each line. The label ending with "original" denotes the total number of embryos imaged at the confocal microscope. The label ending with "OK_orientation" denotes those embryos which have approximately lateral orientation and were analyzed further. The label ending with "final" denotes the number of embryos used in feature detection for extrema and borders. Only T4 to T8 embryos were used. This number includes manually recovered embryos that have more than or less than the normal number of extrema.

Table S7. L2 sequence expanding deletion in *kni* PCR

L2_kni_PCR
TTTTTCTTTTAGTAACAAAAAATAAACAACGAGGGTTTTTGGGGCGACTCCTCCCACTTG
GTTTTTTCGCCGTGTACTTAGACACACACGAATATTCCCCTCATGGCACTAGCCGCATTG
TGGGCCACCGCGAAGCCACCGAAGTGGGTGGCCTCTGCACCACCTAGCTTCTGCCGCTTG
ACTTCCGCATGGGCGGTGGTGGTGTTCGGTGAGTGGGCGGCGGAGTGGGAGGAACTGCTG
CTGCTGGTGGTCAGGCACTCGGCCTCCAGTTGGTAGAACTTCCGGCGCGGAGCCACCTCC
ACTTCTTGATCCTCGGAGCCGCTGTCGTTGAAGCTGTGCACGGAGCTCCGCGAGGTCTTC
ATGCTCAGATCCATGGGTCCTTCTTGAGCGGAAACGGTGGGAGGATGCACTGGTACAACG
CTGGTGGTTTCGCCGCCAATGGAGCAAACCGAAACGGGCGACAAGCTCTGCATCTTGGCC
GCAAAACTCAGCGGGCTGCTGCTAGTCGGAGTGGTTGGCGTGGCTCGGATGGCAATGGGA
TGCAGGAGGCTTGCGGACGACTGATGGCTGTGCACGGAGTGAACATCCTCCTCCAGGCAG
ACATCGATGGGCGACTGGCGGGCCGAGGAGGTGGGCTGCACCACTGGTGGCTGGCTGGCG
GGACTAAAGCGGTTCTGCGACTCAACGGAATCCACGCTCTGGCGGTGCTTGTACATCTCC
TGCCGGTAAGCGGCATCGGCAGCAGAAGCCGCTGCAGCGGCAGCACTTGCATGGTAGCCT
GGGAAGAGGAGGTGTGGGGGCAGCTGGAAGGGCGACTGGTGCGGTACACCGCCCATCATG
CTGAAGAACGGCAGGGCGGCGGATGGGTCGGACAGATAGCTGGGATAGCCCAGCAGAGGC
ATATGTGGATGACGCGGCACCTGCTGCTGCTGCTGCTGCTGATGATGGTGGTGCAAATGG
GCGGCCATGTCCCCAAATCCGGGAGTGTGTGTCGAGCCCACCGGGGAAGAGGCCGACGGG
GCACCACCCACCGATACGCCACCCGCTAATGGAGGCGCCTTGCCCGCCGCTGCGGCGGCC
TGTTCGTGCTCCTGCAGCAGACAATGGATCTTGAACCAGTTGGAGCGACGTCCGTAGCGG
GATCCCCCCTTCGACATGCCCACGTTGTAGCACTTCCTCAAGCGGCACGCCTTGCAGGTG
GTGCGGTTCTTCTTGTCGATGATGCACTTGCCCTCGTTCTTGCACTCGCTGATGGTGCTG
ATGTTGTTGTAAGAGCGGCCAAAGAAGGACTGCAATGGCGAAGGGAAGGACGAATGTGGC
TTAGTTATGTGGTATGCAACTGATTAGCAAAAGAAGAGCAGAAATTCATTCAAGAACTTA
AGGCCAATGGTACAACGATTTGAATCGCCGATGGCCTGTTCCTCGAACATTTCTAAAACT
AACCCCTTGGGGGGTTACGTTCTGCTAATTTATTTGTCAATGGATTTCTTGAGACACAAC
TTACCTTGCAGCCCTCGCAGGTGAAGGCGCCAAAATGGAAGCCCGCCGCCGGCTCACCGC
ACACTTTGCATGTCTGGTTCATCTGGAAGATTGTAAAGAAATCCCGCGTTAGTAAGGGTT
TAATCCACTGGTCGAGAGGTATATGTGTAATCCACAAGTAGGCGAACGGCTCTGGACACT
AACCAAGTTGAACACCATTTTGGAATGATATTTGTTGGAATTTTGAATGCTTTTCTCACG
ATCGCTGTGAGGATCTGCTGTATGATCTGTTCGCTTGGAATGCTGCTGAGCAACTGATGC
TGAGCTCTAGGCGGGCCAGGATATATATAGGAAACTGGGAAAAACTAGACAGGTCTCGCT
CAGAACTTACCTTTTTGCTTTTTTTTGTTGGATGGGCACTAGGTTTATGACGACTTTTTA
ATTGCAGCGATTCGCGATCGTTGCTGCACG

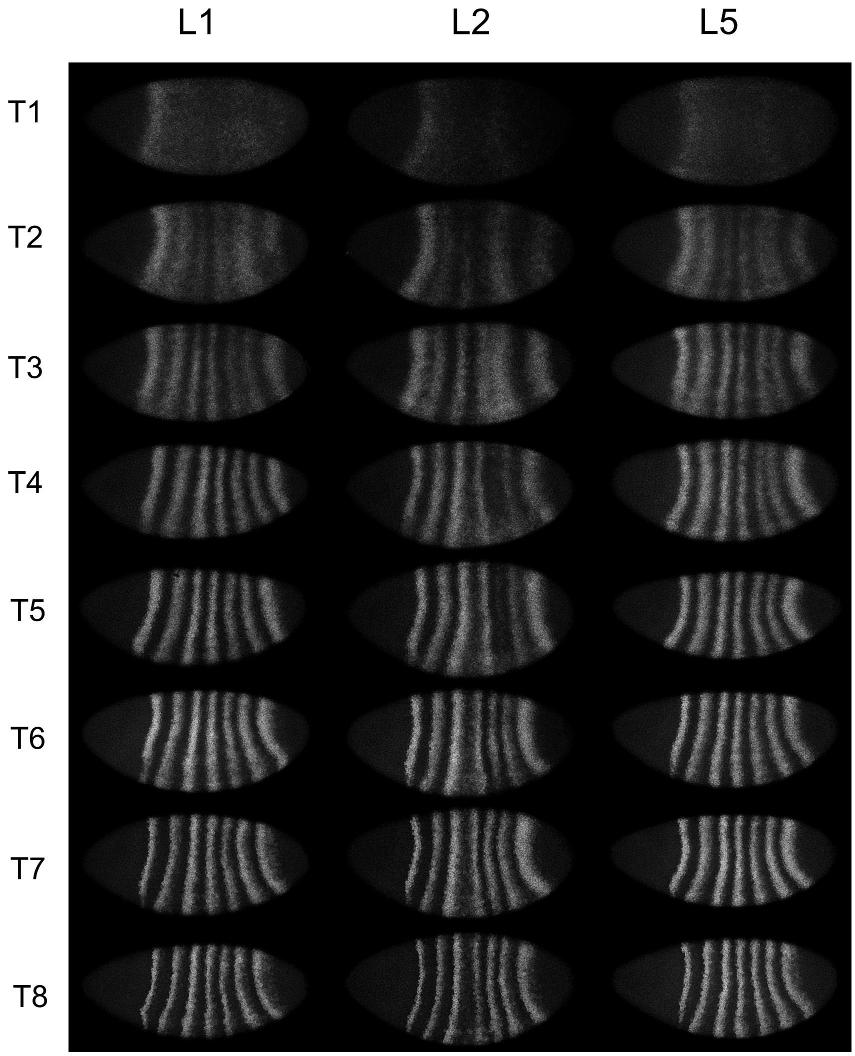

Figure S1. ***eve* RNA expression from 3 lines** Fluorescent *in situ* images of *eve* RNA expression of the same embryos chosen in Figure 1. Anterior is to the left and dorsal is up.

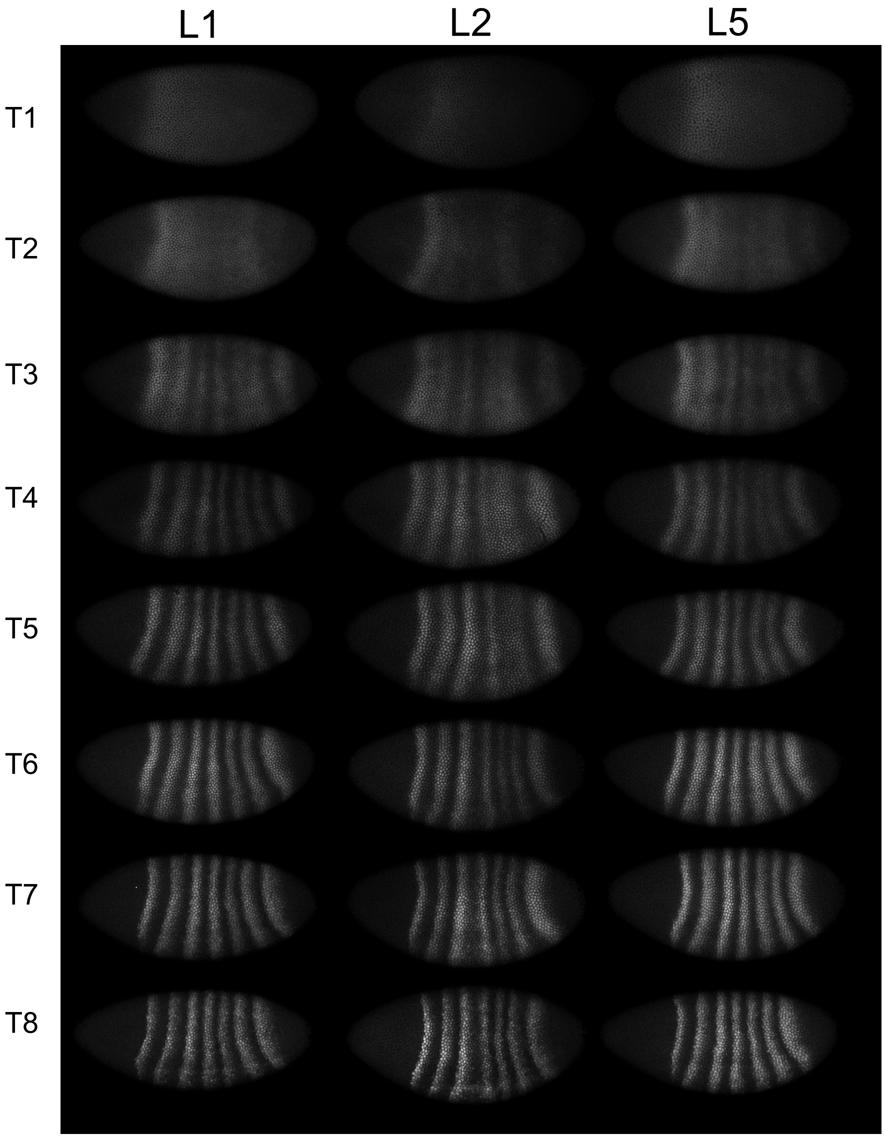

Figure S2. ***eve*** **protein expression from the 3 lines.** Antibody staining for Eve protein of the same embryos chosen in Figure 1.

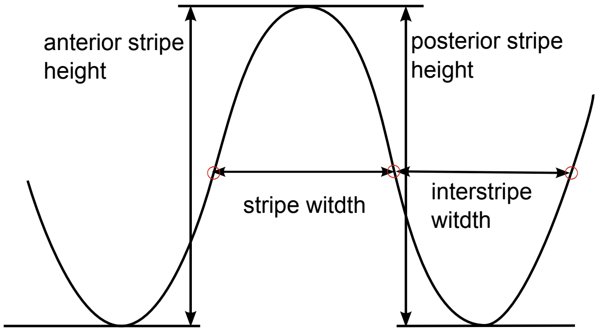

Figure S3. Schematic graph of *eve* stripe features

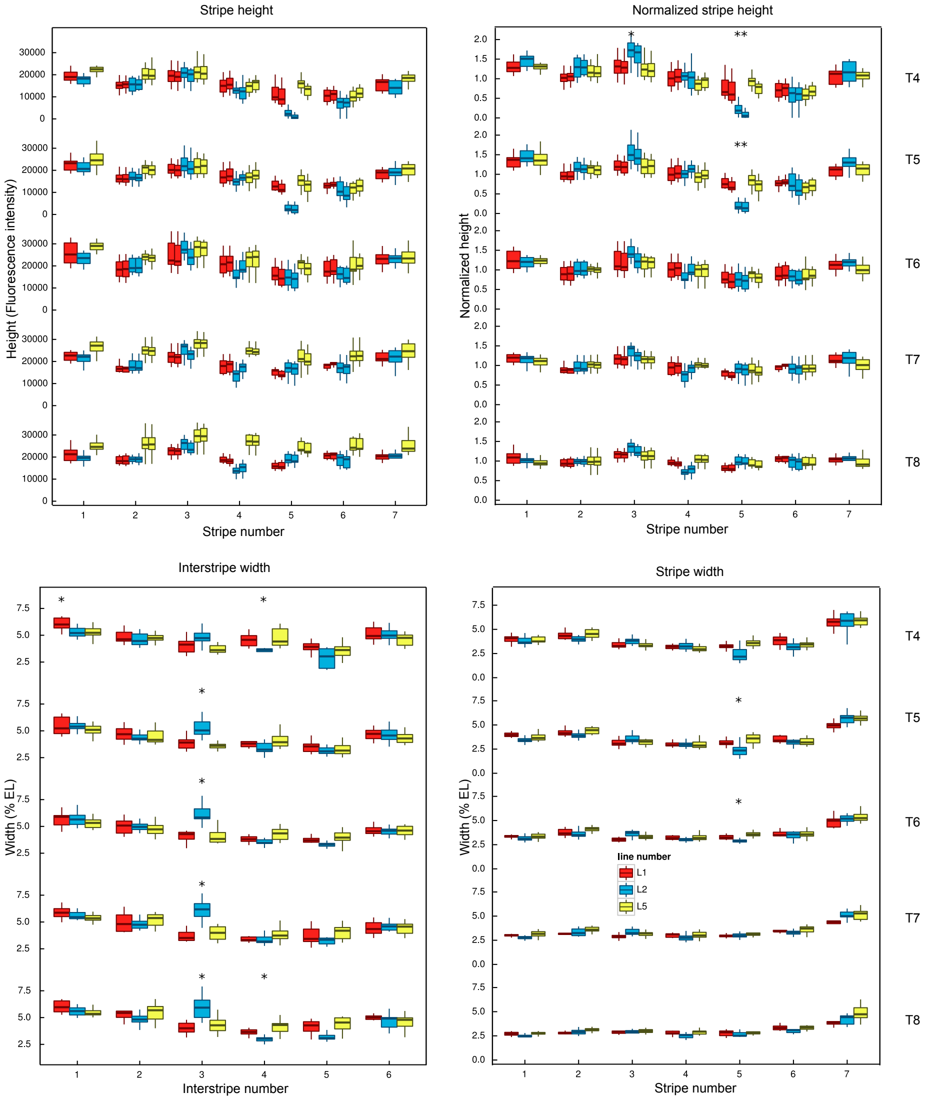

Figure S4. ***eve*** **features for 3 lines** The figure shows stripe height, normalized stripe height, interstripe width, and stripe width for the 3 lines. Red denotes L1, blue L2, and yellow L5. For stripe height, there are two boxes representing each stripe. The one on the left denotes the anterior half of the stripe and the right denotes the posterior half, except the first and last, which have only the posterior half of stripe 1 and anterior half of stripe 7. The asterisks mark the significant difference between one line to the other two lines after Bonferroni correction for each position at each time class (only mark in normalized stripe height, interstripe width and stripe width). Outliers in boxplot are not shown.

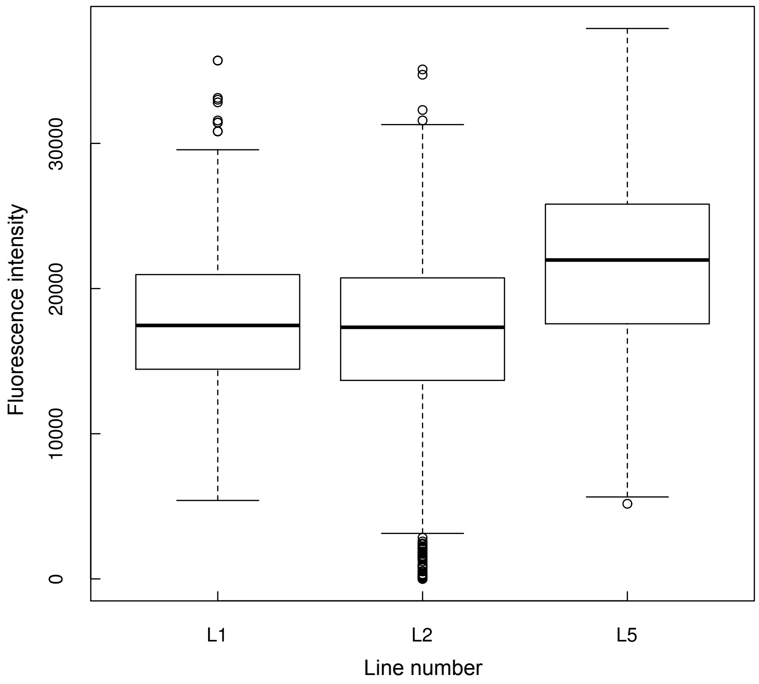

Figure S5. Boxplot of overall stripe height among 3 lines. Boxplot of absolute stripe height in fluorescence intensity for three lines from T4 to T8. L5 is significantly higher than L1 and L2 in overall stripe height. The boundary of box is first quartile, median, and third quartile. The whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Outliers are marked in circles.

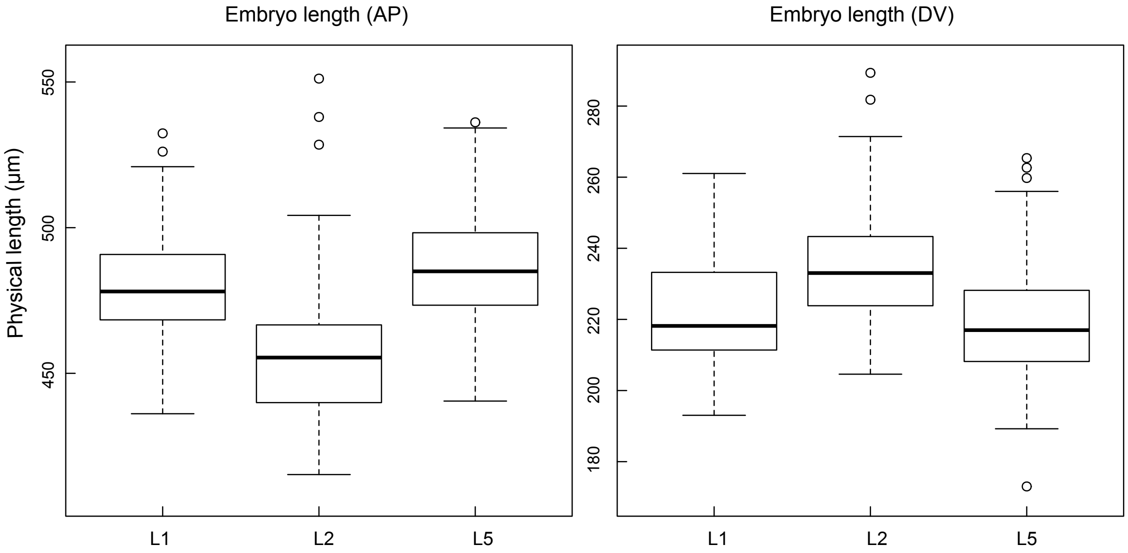

Figure S6. Comparision of embryo size in 3 lines.

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1     TATCCCAAGGCCGCAAAGTCAACAAGTCGGCAGCAAATTTCCCTTTGTCCGGCGAAGTGt  60
1     .......................................................T....  60
1     .......................................................T....  60

61    tttttttttAACCATAACTCGCTGCATTGTTTGGGCCAAGTTTTTCTTCTGCCAAATTGC  120
61    ..........G.................................................  120
61    ..........G.................................................  120

121   GGAGATGATGCGGGGATTATGCGCTGATTGCGTGCAATTATGGACATCCTGCGAGGCCCC  180
121   ............................................................  180
121   ............................................................  180

181   GAGGAACTTCCTGCTAAAGCCTTTCATCCGCCTAAAGAACCCCTTTGTGTCCCGTTCGCC  240
181   ..................T...............C.........................  240
181   ..................T...............C.........................  240

241   GGGAGTCCTTGACGGGTCCTTCGACTATTCGCTTACAGCAGCTTGCGTAAAATTTCATAA  300
241   ............................................................  300
241   ............................................................  300

301   CCCTACGAGCGGCTCTTCCGCGGAATCCCTGGCATTATCCTTTTTACCTCTTGCCAATCC  360
301   ............................................................  360
301   ............................................................  360

361   GTTGGCTAAAAAACGGCTTCGACTTCCGCGTAACTGCTGGACAACAAAGACAAAAAACGG  420
361   ............................................................  420
361   ............................................................  420

421   CGAAAGGACGGCGATTTCCAGGTAGCATTGCGAATTCCGTCAAACTAAAGGACCGGTTAT  480
421   ............................................................  480
421   ............................................................  480

481   ATAACGGGTTTATATGGCCAGAATCTCTGCATCTCCACGACCGCCAGAAGCTGCGTAAAA  540
481   ............................................................  540
481   ............................................................  540

541   CTGCAGGCTCTGTTTTGATTTCTGCAACTTCAGTTAATTGCCCGGGATGGCCAGCAATTG  600
541   ............................................................  600
541   ............................................................  600

601   CCGGCAATTATAAAACAGCGCAGATGTGACTCAGCTTCCATATCTAACTCTATATCTCAT  660
601   ............................................................  660
601   ............................................................  660

661   GCCGAAAATCGagggtggggagcggaggggcggggtgcgtgggtgacttgcctgccaggg  720
661   ............................................................  720
661   ..........T.................................................  720

721   aaagggggcgggggtTCAGCGGGTGATAAATGTGCGTGATTTGGAATGAATGCGCATCGA  780
721   ............................................................  780
721   ............................................................  780

781   TTAAAACCGCAGGGCAATCAATTT  804
781   ........................  804
781   ........................  804
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Figure S7. Alignment of stripe 5 enhancer of the three lines The first row is L1, second is L2 and third is L5.

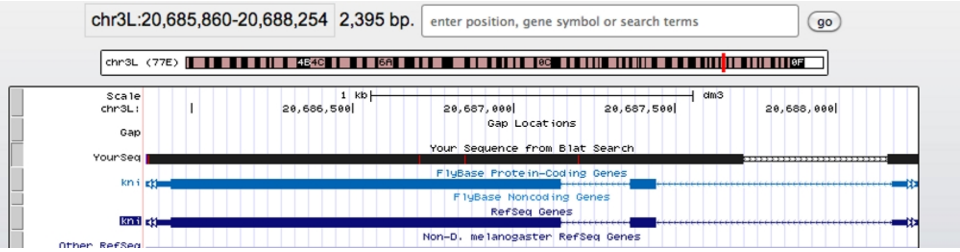

Figure S8. BLAT of L2 showing a deletion in the intron of *kni*