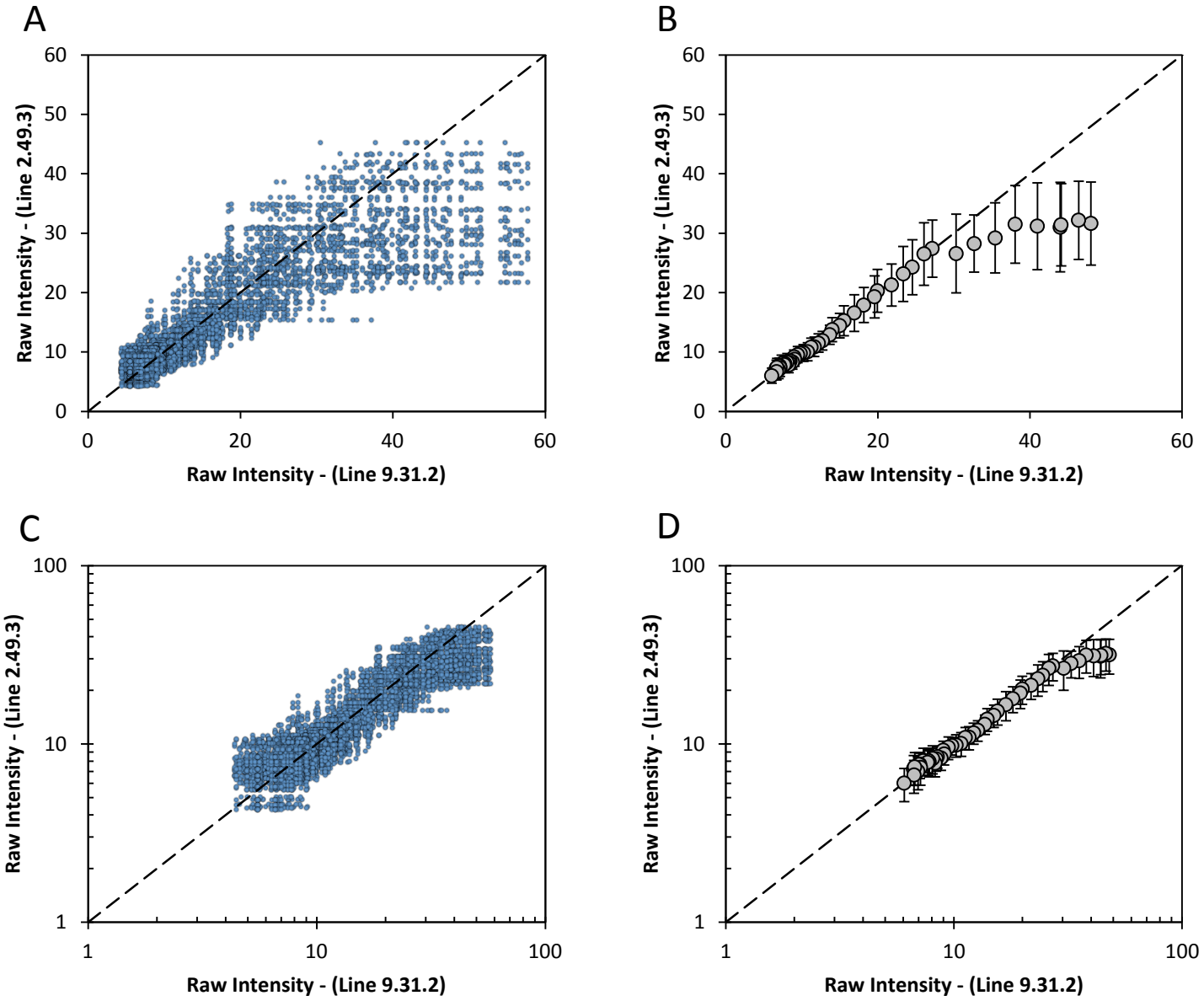

Fig. S1. Bcd profiles in the large and small embryos as a function of relative AP position exhibit a linear relationship. (A-B) Shown are scatter plots of the raw fluorescence intensity values detected in embryos from lines 2.49.3 (*y*-axis) and 9.31.2 (*x*-axis) at their relative positions, plotted against each other. In panel A, all pair-wise combinations of intensity values were used. In panel B, the mean raw intensity values were used, with s.d. shown for the large embryos. (C-D) Shown are the same data plotted on a logarithmic scale. In all panels, an identity line is shown as a reference.

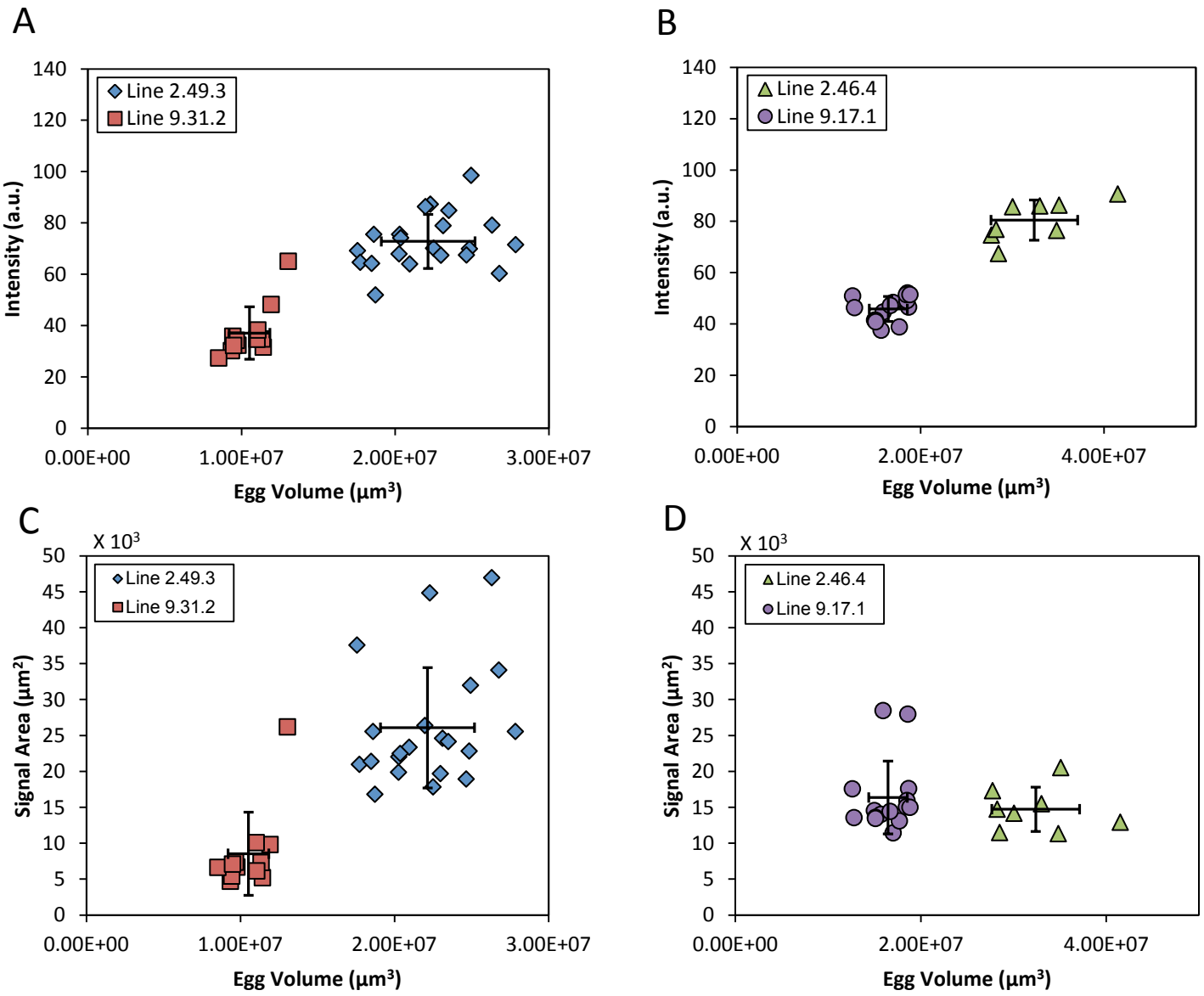

Fig. S2. FISH intensity data plotted against calculated embryo volume. (A-B) Shown are background subtracted, aggregate intensity values for *bcd* mRNA, from lines 2.49.3 (blue) and 9.31.2 (red) (A) and the previously published lines 2.46.4 (green) and 9.17.1 (purple) (B). (C-D) Shown are area sizes of specific *bcd* mRNA signals in embryos from lines 2.49.3 and 9.31.2 (C), and lines 2.46.4 and 9.17.1 (D). In all plots, the volume of each individual embryo was calculated according to the measured egg length *L* and width *W*, assuming a prolate spheroid shape. Error bars are s.d.

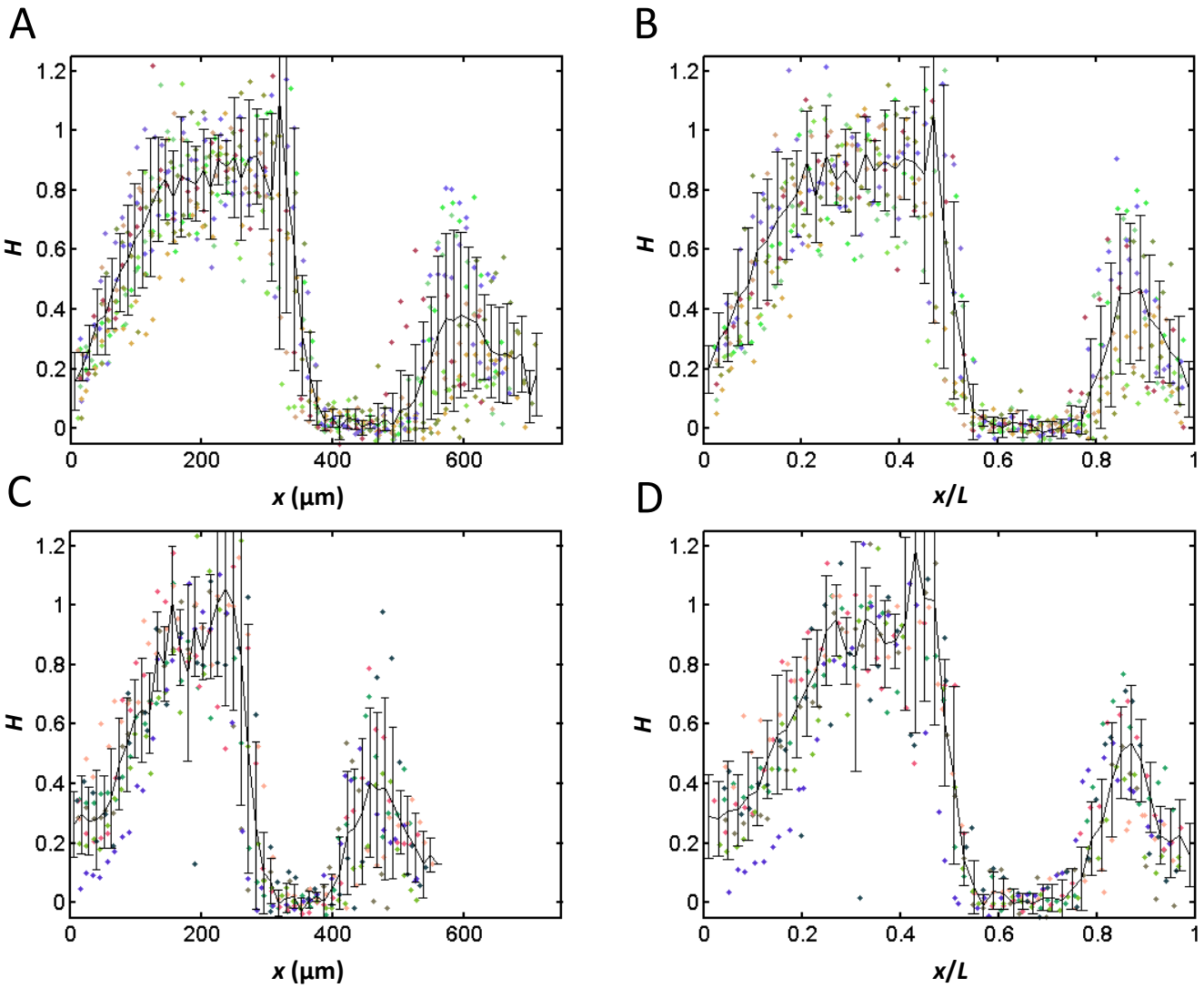

Fig. S3. FISH analysis detecting *hb* mRNA in large and small embryos. Shown are FISH intensity data detecting mature *hb* mRNA in individual embryos from lines 2.49.3 (A,B) and 9.31.2 (C,D), plotted as a function of *x* (A,C) or *x*/*L* (B,D). Also shown in these panels are the mean and s.d. Each color represents data from an individual embryo. *n*=10 and 7 for lines 2.49.3 and 9.31.2, respectively.

Table S1

	Line 2.49.3	Line 9.31.2	Rel Diff (%)	*p*
Egg Length (μm)	666 ± 41	498 ± 30	33.7%	1.5E-13
Egg Volume (μm^3)	2.2E+07 ± 3.0E+06	1.1E+07 ± 1.3E+06	110.5%	1.3E-13
Intensity	7.3E+05 ± 1.1E+05	3.7E+05 ± 1.0E+05	96.5%	1.1E-10
Signal Area (μm^2)	26072 ± 8379	8537 ± 5792	205.4%	3.9E-07
n	21	12		

Table S1. Listing of bcd mRNA FISH data for embryos from lines 2.49.3 and 9.31.2. Shown are the indicated measurements and relative differences between the embryos from these two lines. Also shown are p values from Student's t-tests between the lines.

Table S2

	Egg Length		Egg Volume		Intensity		Signal Area	
	Rel Diff (%)	*p*	Rel Diff (%)	*p*	Rel Diff (%)	*p*	Rel Diff (%)	*p*
Line 2.49.3 vs Line 9.31.2	33.7	1.5E-13	110.5	1.3E-13	96.4	1.2E-10	205.4	3.8E-07
Line 2.46.4 vs Line 9.17.1	21.3	3.7E-09	96.7	2.1E-10	73.0	4.9E-06	-11.2	4.1E-01
Cage 1 vs Cage 7	13.0	3.1E-08	30.4	6.5E-07	100.00	6.6E-07	15.3	1.1E-01
Cage 1 vs Cage 8	14.6	4.5E-07	40.8	9.5E-07	64.1	1.8E-04	16.1	8.7E-02
Cage 1 vs Cage 9	17.1	3.7E-11	40.9	1.2E-09	74.1	1.6E-05	25.8	1.2E-02
Cage 2 vs Cage 7	12.3	7.0E-10	26.5	1.9E-08	128.8	1.5E-08	23.3	7.7E-03
Cage 2 vs Cage 8	13.9	8.9E-09	36.6	5.9E-09	87.8	1.3E-05	24.1	6.9E-03
Cage 2 vs Cage 9	16.3	2.1E-13	36.6	4.4E-12	99.2	1.5E-07	34.6	3.3E-04
Cage 3 vs Cage 7	13.1	1.0E-09	30.2	6.4E-07	116.1	2.4E-07	3.5	6.5E-01
Cage 3 vs Cage 8	14.7	1.5E-08	40.6	9.7E-07	77.3	9.3E-05	4.2	5.2E-01
Cage 3 vs Cage 9	17.2	5.6E-13	40.7	1.2E-09	88.1	2.8E-06	13.0	1.2E-01

Table S2. Listing of bcd mRNA FISH data for embryos from other inbred lines and population cages. Shown are the indicated measurements and relative differences. See Table S1 legend for details. The comparisons made here were between all possible pairs. All comparisons were made only for experiments that had been performed side by side. See Fig. 4 and Fig. S2 for graphic presentations of data.

Table S3

Gene	Amplification Primers	Sequencing Primers
bcd	5‘-ACAGGCAGCTGGTGCAAATG-3‘ (f)	5‘-GGACTAGACCTAACTTTCTACGCG-3'
	5‘-TCAGGCATGAGTCCACAACC-3‘ (r)	5‘-CCCTGAAAACTAAGGGCT-3'
	5‘-GACCCTTCAAAGGCTCCAAG-3‘ (f)	5‘-GTGATGGTATTGCTGCTGCT-3'
	5‘-CAGCTTTGCCGTACTGTTCG-3' (r)	5‘-CGGTGTGAGTGCAACAGGTT-3'
bcd 3' UTR	5 ‘-TCGCAGTTTGCCTACTGCTT-3‘ (f)	5‘-TCGCAGTTTGCCTACTGCTT-3'
	5‘-GAAAGGGACGGAAATATGGG-3' (r)	
exu	5‘-TTACGGATCCACCAGAACTG-3‘ (f)	5‘-TTATGGTTACGGCAGGTGGA-3'
	5‘-ATCTAGTGAAAGCGGTTCGC-3‘ (r)	5‘-ATATCCAGCTCCAGGACATC-3'
		5‘-AGACCACTCTGTACCATCGT-3'
		5‘-CTCAAGCCAGTTGAGGAAGT-3'
stau	5‘-TTCGCCTCTGTTCAGTTTCG-3‘ (f)	5‘-TTCGCCTCTGTTCAGTTTCG-3'
	5‘-TGCTGGAAGCTGTTCAAGTC-3‘ (r)	5‘-GTCACGATGGTCAGGAACTC-3'
	5‘-ATTTTCAACGTAGGGCAGGG-3‘ (f)	5‘-GACTTGAACAGCTTCCAGCA-3'
	5‘-TGATCCCTCTTCTTTGCTGC-3‘ (f)	5‘-TGCAAGACCTTCACGGTGAC-3'
	5‘-AGCACTTGAGTAGCAGCAAC-3' (r)	5‘-ACATGGTTTCCTCGATAGCC-3'
	5‘-GGGGATGATAACCATTTCCG-3‘ (f)	5‘-CGCCTGGCTCATGTAATAGT-3'
	5‘-TCTTGGTCTTGGTTTGGGTC-3' (r)	5‘-GGGGATGATAACCATTTCCG-3'
swa	5‘-GCTGAAGCTCTGCGTAATTG-3‘ (f)	5‘-CAAAGCAGCCACTGTCAATG-3'
	5‘-ACATCCGGCGTTAGTGCATT-3' (r)	5‘-TGAATGGTGTCATGGCTCTG-3'
		5‘-GGATCCTTTGCTGCTGTTGT-3'
		5‘-TTCGTGTAGGCCGAATGGAT-3'
Vps36	5‘-GCCGACAAAGTCAAAGCAGA-3’ (f)	5‘-CAGTCTTGTCATTGCGATGG-3'
	5‘-GCCACTTATCGATAGTCAGC-3‘ (r)	5‘-CCTTGCTCCTAAGGGGTAAT-3'
		5‘-TGATTATGCGAGTCTTGCGG-3'

Table S3. Listing of all primers used for PCR amplifications and DNA sequencing. For PCR primers: f, forward; r, reverse.

Table S4

Gene	Chromosomal Location	Nucleotide Changes	Amino Acid Changes	Flybase ID
bcd	3R:2583672	(G→A)	V279M	FBpp0081168
	3R:2582607	(A→G)	N/C	
bcd 3′ UTR	N/C		N/A	
exu	2R:16556761	(G→A)	N/C	FBpp0085555
	2R:16556701	(A→G)	N/C	
	2R:16556437	(C→T)	N/C	
stau	2R:14010314	(G→C)	G84A	FBpp0085962
	2R:14009928	(C→G)	N/C	
	2R:14009574	(C→T)	N/C	
	2R:14009580	(T→C)	N/C	
	2R:14009385	(C→T)	N/C	
	2R:14009232	(G→A)	N/C	
	2R:14009280	(C→G)	N/C	
	2R:14009184	(C→T)	N/C	
	2R:14009112	(A→T)	N/C	
	2R:14008943	(C→A)	A547D	
	2R:14008665	(A→G)	N/C	
	2R:14008578	(C→A)	N/C	
	2R:14008590	(G→T)	N/C	
	2R:14008513	(C→A)	P684T	
	2R:14008517	(C→T)	T685M	
	2R:14008527	(C→A)	N/C	
	2R:14008278	(G→A)	N/C	
	2R:14008119	(A→T)	N/C	
	2R:14007636	(T→A)	N/C	
	2R:14007504	(T→C)	S797C	
	2R:14007522	(C→T)	N/C	
	2R:14007432	(C→T)	N/C	
	2R:14007441	(C→G)	N/C	
	2R:14007450	(G→A)	N/C	
	2R:14007394	(C→G)	L1021V	
swa	N/C		N/C	
Vps36	3L:13504804	(C→T)	I145T	FBpp0075560
	3L:13504878	(A→G)	K221E	
	3L:13504938	(C→T)	N/C	
	3L:13505058	(G→A)	A356T	
	3L:13505404	(T→C)	N/C	
	3L:13505628	(G→A)	N/C	

Table S4. Listing of genes sequenced and deviations identified. Chromosome locations are based on the genomic sequence data in FlyBase and the predicted aa changes are according to the annotated polypeptides of the indicated FlyBase ID. N/A, not applicable; N/C, no change.