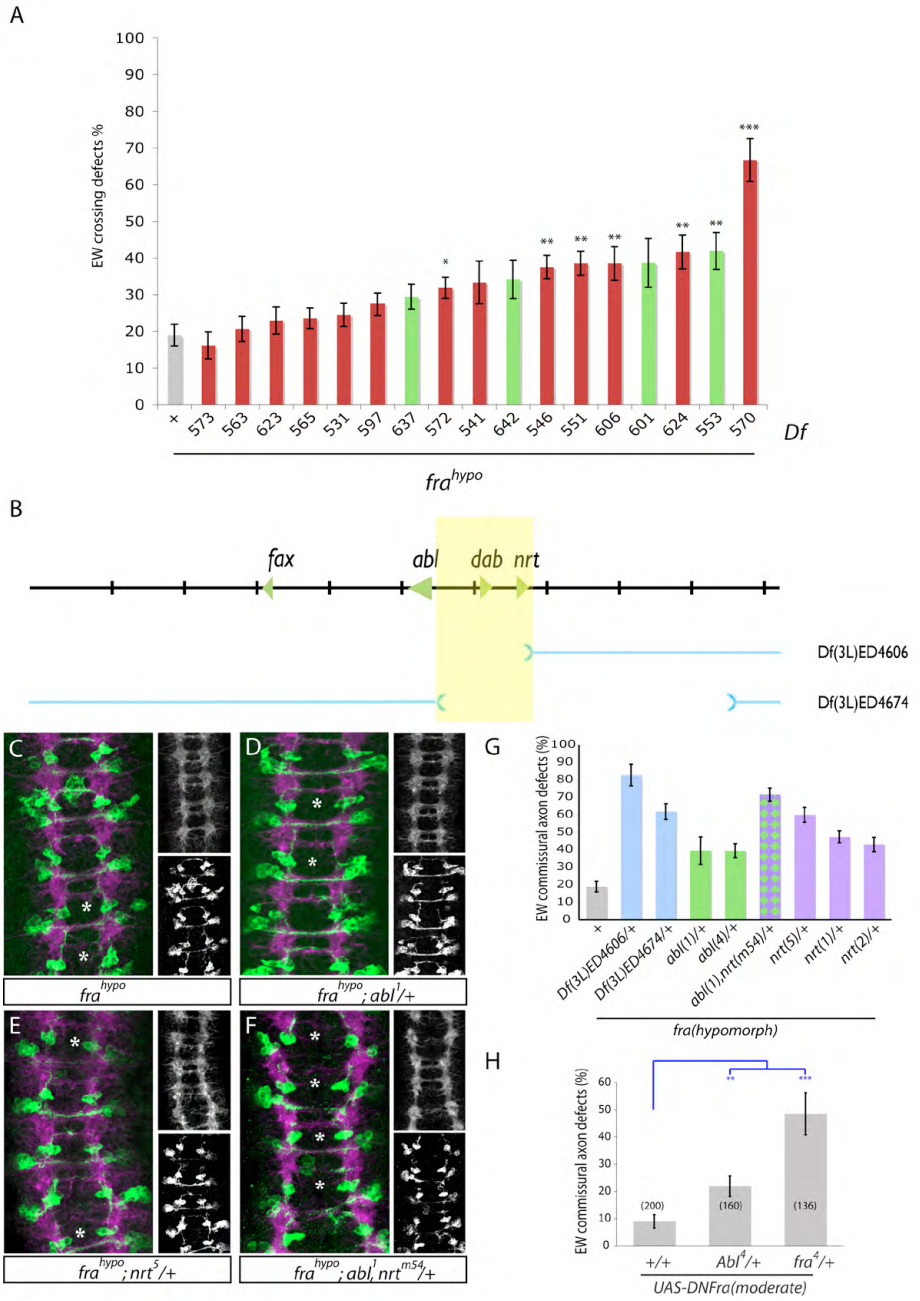

Fig. S1. A deletion-based screen for genes that promote commissural axon crossing. Secondary screen for intervals identified in the primary screen (see also supplementary material Table S1). Details of the primary screen are described in the Results and in supplementary material Table S1. (**A**) Secondary screen results. Seventeen different heterozygous deletions, identified in the primary screen were introduced into *fra3/fra6* mutants, and we scored the EW crossing phenotype. Each deletion is indicated by a stock number (for details on each Df, see Table S1). 'x' indicates the control background. Data is represented as the percentage of non-crossing segments. Deletions are colored according to whether the were originally identified as enhancers (red) or suppressors (green). Significantly enhancing deletions are indicated. None of the intervals initially identified as suppressors reduced crossing defects in *fra* hypomorphs, indicating that this screen was probably only specific for enhancers in the *fra* pathway. Error bars indicate s.e.m. Data were analyzed using Kruskal Wallis ANOVA followed by Wilcoxon rank-sum test. A post-hoc holm correction was applied to account for multiple comparisons. (**B**) Schematic showing the minimally mapped interval for the enhancer identified in the primary screen, Df(3L)ED223. The breakpoints of the two deletions included in this figure are indicated in blue. Df(3L)ED223 (not pictured) does not include *fax,* but includes *Abl.* (**C-F**) Micrographs showing four embryonic abdominal segments in stage 15 embryos of the indicated genotypes. CNS axons are labeled with mAb BP102 (magenta). EW axons are labeled with anti-GFP to visualize TauMycGFP expressed in these neurons. (C) A *fra*^{hypo} embryo. Mild EW defects (asterisks) and defective commissures can be observed (top-right panel). (D) *fra*^{hypo}*; Abl1*/+ embryo, EW defects are enhanced (asterisks). Commissures are not obviously affected (see top-right panel). (E) A *fra*^{hypo}*; nrt5*/+ embryo. Both EW defects (asterisks) and commissure formation are affected. (F) A *fra*^{hypo}*; Abl1 nrtm54*/+ embryo. Commissural defects are as severe as those seen after a deletion of these two genes. (**G**) Quantification of EW crossing defects. Both *Abl* and *nrt* heterozygous mutations enhance EW crossing defects in *fra*^{hypo} mutants. (**H**) Quantification of EW crossing defects in stage 15-16 embryos of the indicated genotypes expressing DN-Fra with *eagle*-Gal4. Heterozygosity for *Abl* (middle) or *fra* (right) enhances crossing defects. **P*<0.05, ***P*<0.01, ****P*<0.001.

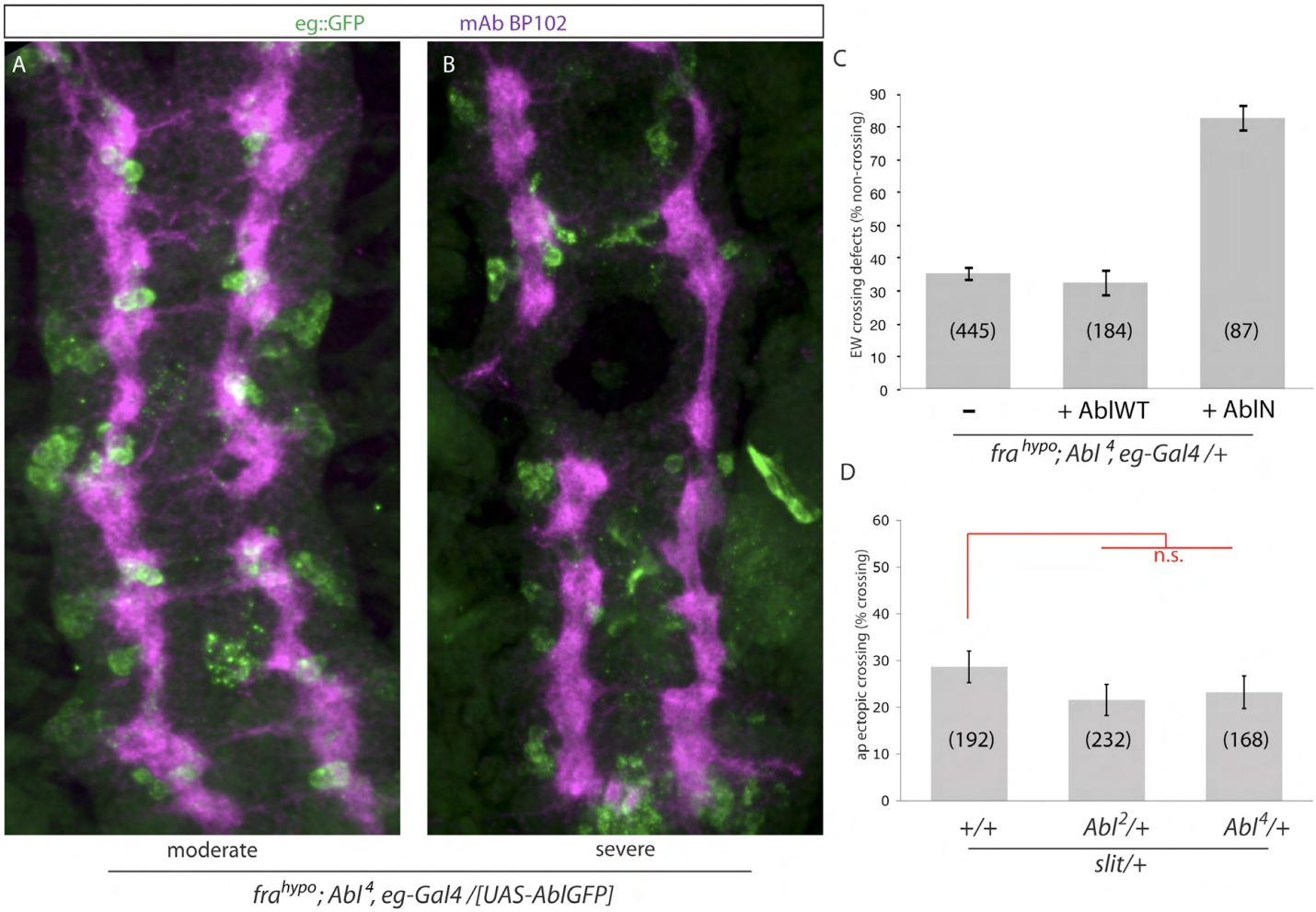

Fig. S2. Patterning defects in *fra* mutant embyros bearing WT Abl transgenes. (**A**,**B**) Representative stage 16 embryos bearing WT-Abl transgene in *fra* mutants showing patterning defects as evidenced by severe midline crossing defects and aberrant *eg*-Gal4-positive cell body positions and numbers. The genotype of these embryos is *fra3/fra6; Abl4,eg-Gal4 / UAS-Abl-GFP.* We have observed this phenotype in multiple *fra* allelic combinations and with different *Abl* transgenes inserted at multiple loci, even in the absence of Gal4-driver. Abl^{KN} does not result in these defects. We attribute this phenomena to basal expression of UAS constructs in the absence of driver, though we have been unable to detect this presumed expression through immunostaining. (A) A moderate patterning phenotype. Note the near absence of commissures and variable EW cell number. For comparison without *Abl* transgene, see Fig. 1B and supplementary material Fig. S1D. (B) Severe patterning phenotype. Note the absence of commissures and longitudinal connectives. EW neurons cannot be identified or are located in aberrant positions. (**C**) Quantification of rescue f*ra3/fra6; Abl4,eg-Gal4* / + using Abl-WT or Abl-N. Neither Abl-WT nor Abl-N rescue midline crossing defects in this genetic background. For this quantification, we attempted to use only the subset of embryos displaying apparently mild or no patterning defects. We may have missed more subtle defects, thus we caution that little can be concluded from these data. (**D**) Quantification of *ap* axon ectopic crossing in stage 17 *slit*/+ embryos of the indicated genotypes. Heterozygosity for *Abl* does not modify *ap* ectopic crossing in *slit*/+ embryos. Error bars represent s.e.m. Number of segments scored is shown in parentheses. ***P*<0.01, ****P*<0.001.

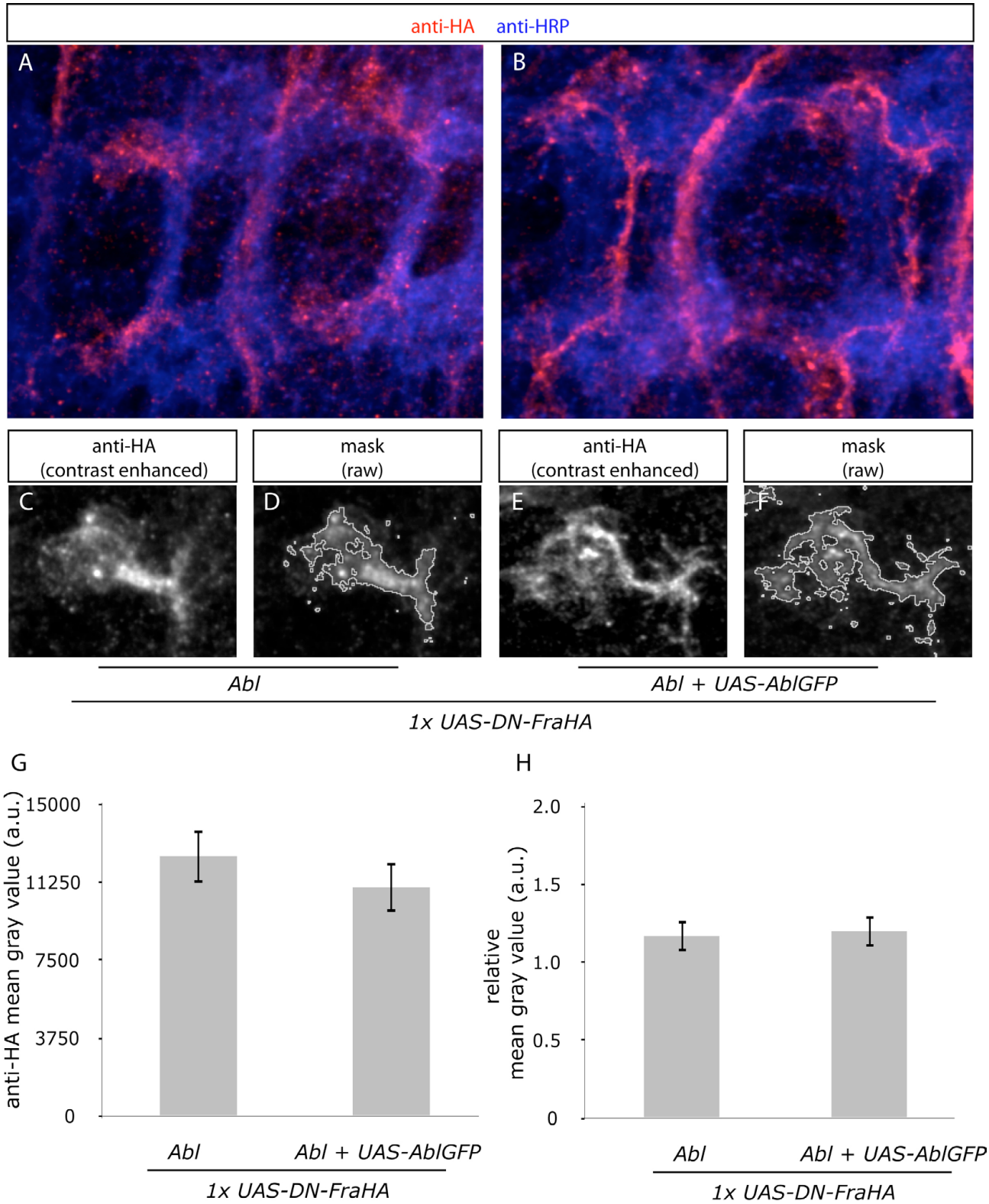

Fig. S3. Abl-GFP overexpression does not affect DN-Fra levels. (**A-F**) Stage 14 *Abl*^{2} embryos expressing DN-Fra-HA (A,B) or co-expressing DN-Fra-HA with Abl-GFP (B) in EW neurons using *eg*-Gal4. DN-Fra-HA is visualized with anti-HA immunostaining (magenta) and axons are labeled with anti-HRP (blue). (A) *Abl*^{2} embryo expressing DN-Fra. (B) *Abl*^{2} embryo rescued with Abl-GFP. DN-Fra levels are similar to panel A. (C-F) Single EW neuron cluster showing DN-Fra expression level using anti-HA immunostaining. Contrast is identically enhanced in C and E to show axonal localization, whereas D and F show raw pixel intensities and corresponding mask used for quantification (outlined). (**G**) Quantification of raw pixel intensity values for five EW neuron clusters of each of the indicated genotypes using anti-HA immunostaining. (**H**) Quantification of raw pixel intensity values for five EW neuron clusters of each of the indicated genotypes using anti-HA immunostaining normalized to anti-HRP immunostaining. No significant differences are identified in this quantification. Quantification was performed using ImageJ. Error bars represent s.e.m.

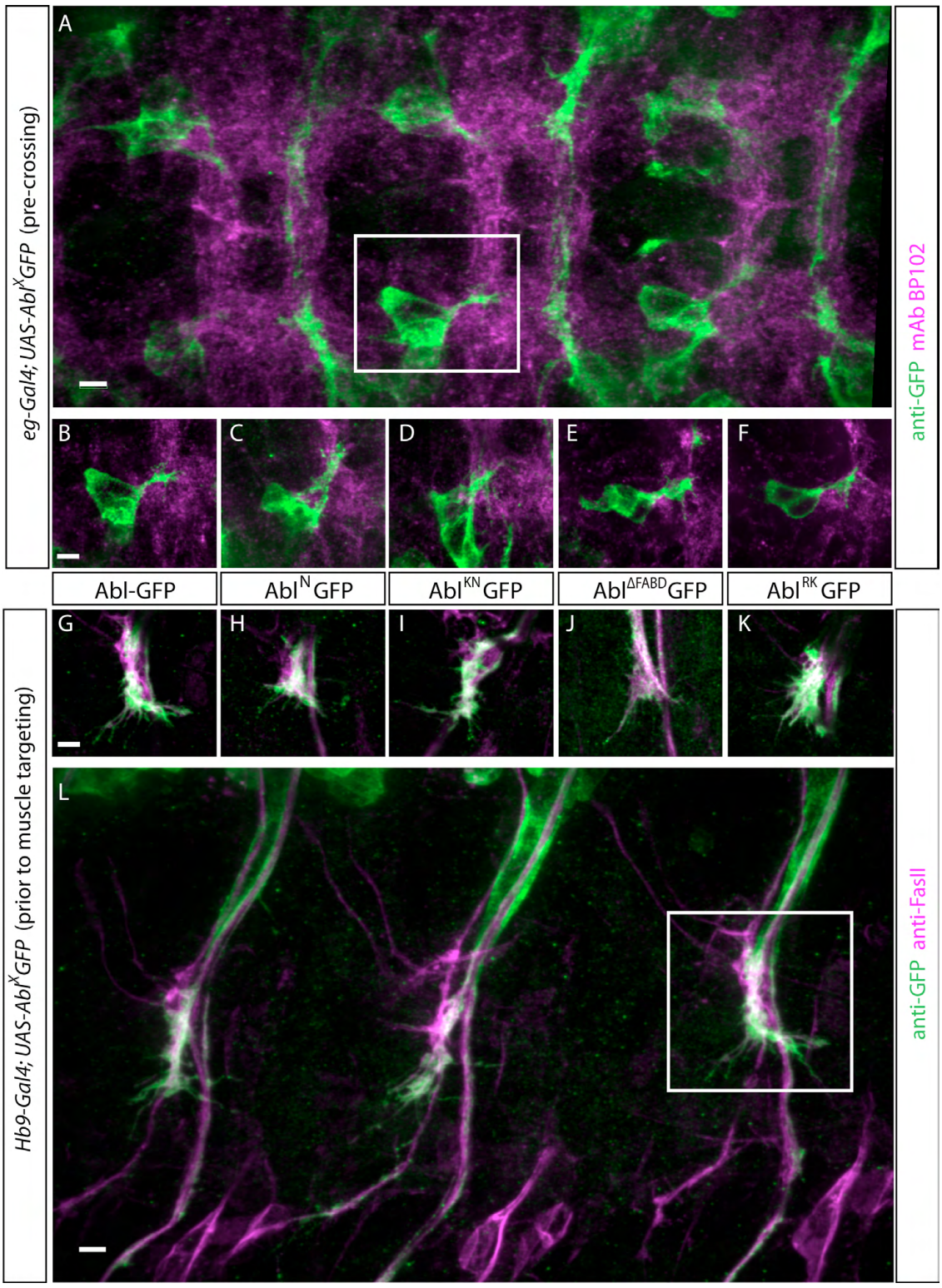

Fig. S4. Expression and localization of Abl transgenes used in this study. (**A-F**) Age-matched stage 13 embryos showing Abl:GFP (or the indicated mutant constructs) expression in *eg*-Gal4 positive neurons prior to midline crossing. Anti-GFP (green) shows Abl localization and expression, whereas mAb BP102 (magenta) shows all CNS axons. Anterior is to the right. Note the cell body and growth cone localization of all constructs. A shows three embryonic segments of an embryo expressing Abl-GFP. The EW cluster shown in B is boxed for reference. (B-F) Single EW neuron cluster expressing each of the indicated constructs. (**G-L**) Representative age-matched stage 15 embryos expressing Abl-GFP in *Hb9-Gal4*-positive motoneurons during a time when they are entering the ventral muscle field, but have not yet reached muscle targets. Anti-GFP (green) shows Abl localization and expression, whereas anti-FasII (magenta) shows all motor axons. Anterior is to the right, dorsal is down. Note that all constructs, except AblΔFABDGFP, appear to be expressed at similar levels and localize to growth cones and filopodia. AblΔFABDGFP shows reduced expression in growth cones and filopodia. L shows three embryonic segments of an embryo expressing Abl-GFP in motor axons. The RP growth cone shown in G is boxed for reference. Scale bars: 5 μm.

Table S1. List of enhancers and suppressors from primary screen- UAS-FraDeltaC #4

#	Df	Elav::DeltaC	Penetrance (% comm)	*n*	Eg::DeltaC	Penetrance upper vs lower (%NC)		*n*	Control (EW)
531	DF(3L)ED201	N/A	N/A	N/A	**suppressor**	**60.2%**	61.4%	11	90%
541	Df(3L)ED208	**enhancer**	N/A	N/A	N/A	84.7%	95.8%	9	
542	Df(3L)Exel6098	N/A	N/A	N/A	**suppressor**	**75.0%**	82.5%	15	
546	Df(3L)Exel6104	**enhancer**	N/A	N/A	N/A	N/A	N/A	N/A	
550	Df(3L)XAS96	**enhancer**	**31.3%**	16	N/A	88.0%	100.0%	11	
551	Df(3L)XDI98	**enhancer**	**46.2%**	19	N/A	88.0%	100.0%	10	
552	Df(3L)RM5-1	**enhancer**	**25.0%**	16	N/A	88.0%	100.0%	11	
555	Df(3L)ED4408	**enhancer**	11.8%	17	N/A	88.0%	100.0%	7	
563	Df(3L)ED4483	**enhancer**	**18.2%**	11	N/A	N/A	N/A	N/A	
565	Df(3L)ED4502	**enhancer**	15.4%	13	N/A	84.7%	94.4%	9	
570	Df(3L)ED223	**enhancer**	**17.6%**	17	N/A	88.0%	100.0%	8	
572	Df(3L)ED4685	**enhancer**	13.0%	23	N.A	88.0%	100.0%	10	
597	Df(3R)Antp-X1	**enhancer**	**17.6%**	17	N/A	88.0%	100.0%	11	
601	Df(3R)Exel6151	**suppressor**	**-10.5%**	19	**suppressor**	**78.6%**	85.7%	7	
604	Df(3R)GB104	**enhancer**	15.8%	19	N/A	88.0%	100.0%	7	
623	Df(3R)Exel7326	**enhancer**	16.7%	21	N/A	88.0%	100.0%	12	
624	Df(3R)Exel8162	**enhancer**	15.0%	20	**suppressor**	**82.3%**	90.6%	12	
637	Df(3R)Exel6191	N/A	0.0%	20	**suppressor**	**74.0%**	81.3%	12	
640	Df(3R)Exel6200	N/A	0.0%	19	**suppressor**	80.7%	89.8%	11	
642	Df(3R)Exel9056	**suppressor**	**-10.0%**	20	**suppressor**	**64.6%**	67.7%	12	
643	Df(3R)D605	**suppressor**	**-20.0%**	30	N/A	86.3%	97.5%	10	

Table S1. A summary of primary screen results. The primary screen was a qualitative screen for genomic deletions that enhance or suppress the DN-Fra commissural guidance phenotype. We estimate that this screen covered ~52% of the third chromosome (3L: 44 Dfs, 75% covered, 13.8% double coverage, mean interval 440 kb; 3R: 31Dfs, 36.6% covered, 4% double coverage, mean interval 280 kb), which accounts for 22.5% of the *Drosophila* genome. Twenty-one deletions were identified in the primary screen as either enhancing or suppressing commissural guidance defects. Enhancers (red) and suppressors (green) are were identified in either the pan-neural screen (Elav::DeltaC) or in the EW screen (Eg::DeltaC), or both. N/A indicates that a deletion did was not identified in a particular screen, or was not screened. For Elav screen, we sorted embryos phenotypically into three classes (class 1, 'mild'; class 2, 'moderate'; class 3, 'comm'). Most embryos in this background fall into class 2. enhancers are expressed as the percentage of embryos with severe commissural defects (% comm). A negative value means that a percentage of embryos showed a milder phenotype. For EW crossing defects, embryos were classified in eight categories, based on the number of EW non-crossing segments (of eight segments). The strongest class included embryos with both seven and eight non-crossing segments, hence the upper and lower penetrance in the table.

Table S2. Primer sequences

Oligo name	Sequence (mutated bases capitalized)
AblFL sense (Not1)	5'- cac cgc ggc cgc tgg caa atg ggg gct cag cag ggc aag gac -3'
AblKinase-GFP Sew AS	5'- agc tcc aat cac tag tcc aaa cat gtg ctc cag cgc atg gtg -3'
AblKinase-GFP Sew Sense	5'- gga cta gtg att gga gct agc atg gtg -3'
AblCSense-EcoR1	5'- cac cga att cca gga atc gtc cat cac cga ag -3'
GFP 3' Xba-long (AS)	5'- acg ttc gag gtc gac tct aga tta ctt gta cag ctc gtc -3'
Abl K417N Sense	5'- acg gtg gct gtt aaC acg ctc aag gag gac acc atg gc -3'
Abl K417N AS	5'- tcc tcc ttg agc gtG tta aca gcc acc gta ttg cca tac c -3'
Abl W243K Sense	5'- tcg ggg gag AAg tgc gag gcg cac tcg gac tcc gga aac g -3'
Abl W243K AS	5'- cgc ctc gca cTT ctc ccc cga ttt gtt gta gct aag tat gc -3'
Abl R297K Sense	5'- ttc ctg gtc AAA gaa agt gaa agt tca ccg ggt caa agg agc -3'
Abl R297K AS	5'- ttc act ttc TTT gac cag gaa act gcc att gat tcc gga gc -3'
Abl DeltaFABD sense	5'- cag aag cca cag gga cta gtg att gga gct agc atg g -3'
Abl deltaFABD AS	5'- aat cac tag tcc ctg tgg ctt ctg ctc gta cag cg -3'