Supporting Information

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SI RNAi Against *svb* in *D. virilis* Embryos

We tested whether *svb* is required for trichome morphogenesis in embryos of *D. virilis* by delivering 27 mer Dicer-substrate RNAs (DsiRNAs) (1) to 0–2-h-old embryos of *D. virilis*. We designed two DsiRNAs against different regions of the *D. virilis svb* mRNA (Fig. S3). DsiRNAs were chemicaly synthesized by Integrated DNA Technologies, Inc. We also purchased a DsiRNA control targetting the EGFP gene as a negative control. DsiRNA was either injected at 0.2 μM in 0.1 mM Sodium Phosphate pH 7.8, 5 mM Potassium Chloride or delivered biolistically using a BioRad PDS-100/He system. For biolistics, 1 μm gold particles were coated with DsiRNA as follows. Sterile beads were prepared in 50% (vol/vol) glycerol in 1.5 mL Eppendorf tubes following the manufacturer's instructions. Beads were pelleted, and glycerol was removed and replaced with 50 μL 50 μM DsiRNA. The bead suspension was placed on an Eppendorf Thermomixer at 1,400 rpm, and the top of the Eppendorf was opened. Twenty-five μL of 0.1 M spermidine was added to the open tube. Eighty μL of 1 M $CaCl_2$ was added, one drop at a time, to the tube. The tube was capped and left mixing at 1,400 rpm for 5 min. The tube was then placed on ice for at least 15 min. The gold particles were pelleted, washed twice with 300 μL of freshly prepared 70% ethanol, and resuspended in 60 μL absolute ethanol. Tubes were wrapped in parafilm and stored at –80 °C until use. Embryos were dechorionated and shot with gold particles using a vacuum of 26-inch Hg, a target distance of 6 cM, and a Helium pressure of 1,100 psi. Embryos were stored in a humidified chamber for 24–48 h to allow hatching. Both injection and biolistics of DsiRNA produced comparable results (Fig. S4).

1. Kim DH, et al. (2005) Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy. *Nat Biotechnol* 23(2):222–226.

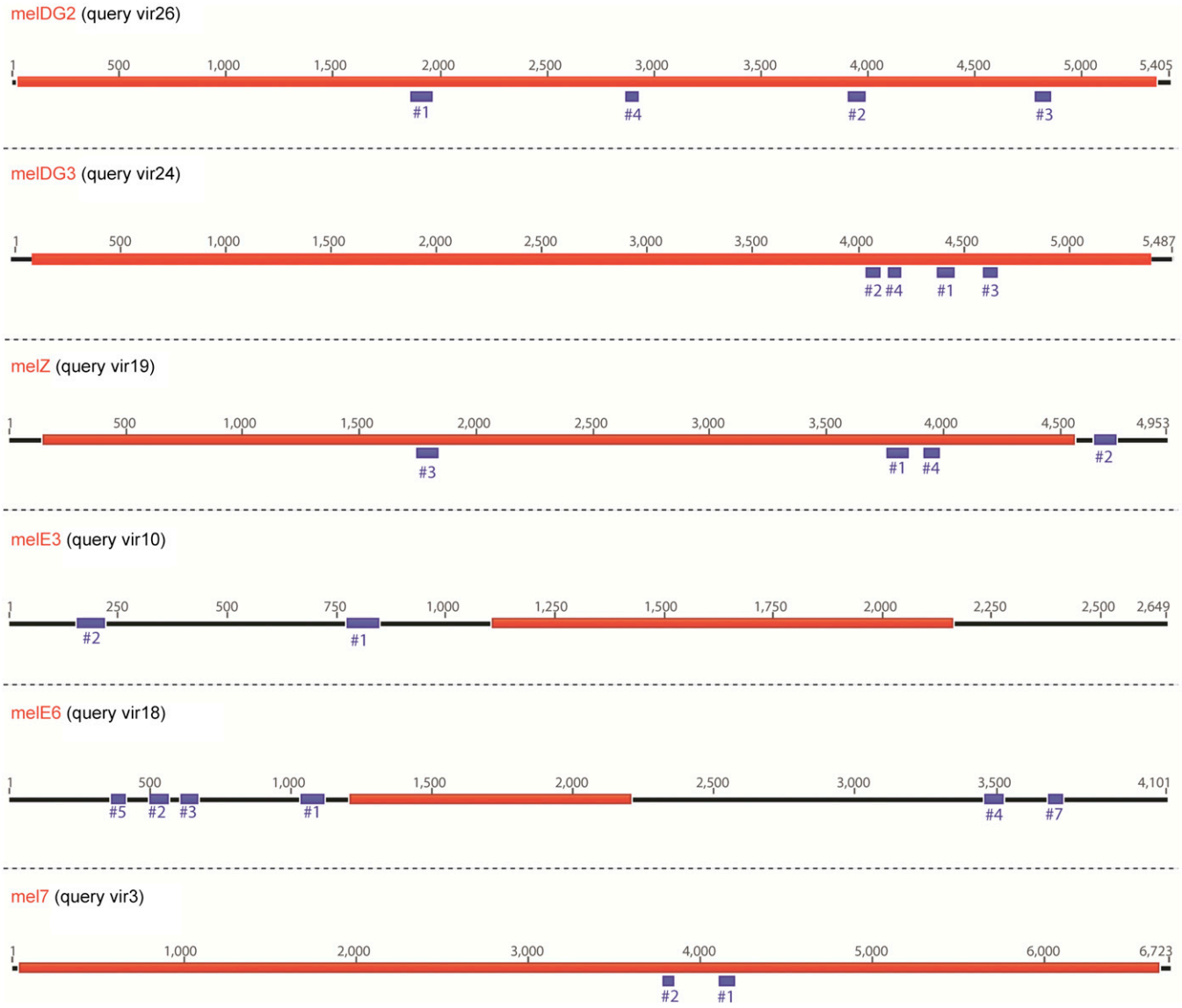

Fig. S1. Significant BLAST hits within and around the *D. melanogaster svb* enhancers. The BLAST hits were obtained with FlyBase BLAST tool (default parameters), using the full sequence of *D. virilis svb* enhancers as queries (see label above each scheme). Significant BLAST hits (E-value < 0.01) were mapped onto the *D. melanogaster svb* locus. The regions in red demarcate the *D. melanogaster* enhancers. The black lines represent flanking DNA. Regions corresponding to significant BLAST hits are marked with blue boxes. The number of each HSP (high-scoring segment pair) is indicated below each blue box. Sequence length is indicated in base pairs.

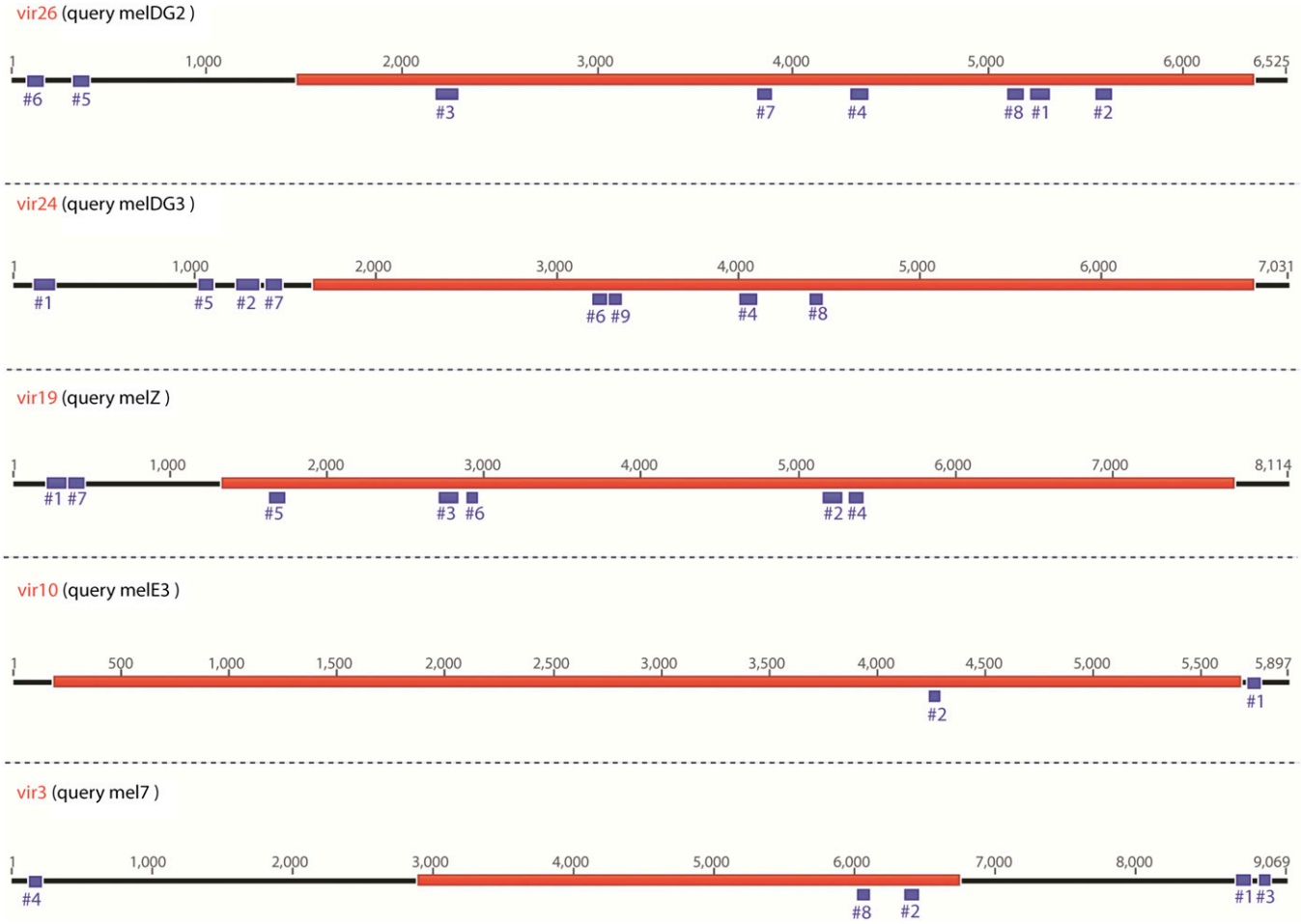

Fig. S2. Significant BLAST hits within and around the *D. virilis svb* enhancers. The BLAST hits were obtained with FlyBase BLAST tool (default parameters), using the full sequence of *D. melanogaster svb* enhancers as queries (see label above each scheme). Significant BLAST hits (E-value < 0.01) were mapped onto the *D. virilis svb* locus. The regions in red demarcate the *D. virilis* enhancers. The black lines represent flanking DNA. Regions corresponding to significant BLAST hits are marked with blue boxes. The number of each HSP is indicated below each blue box. Sequence length is indicated in base pairs. No significant similarities were found using mel*E6* as query.

Fig. S3. Target locations of the two DsiRNAs designed against the *D. virilis svb* cDNA. The sequence for the *D. virilis svb* cDNA is National Center for Biotechnology Information Reference Sequence XM_002057882. The sense strand sequences of the DsiRNAs are as follows: svb3—5′GCAACAACAACAACAACAACAGCAA; svb5—5′CGAACAATTTGCTCGGTCAGCTGCC.

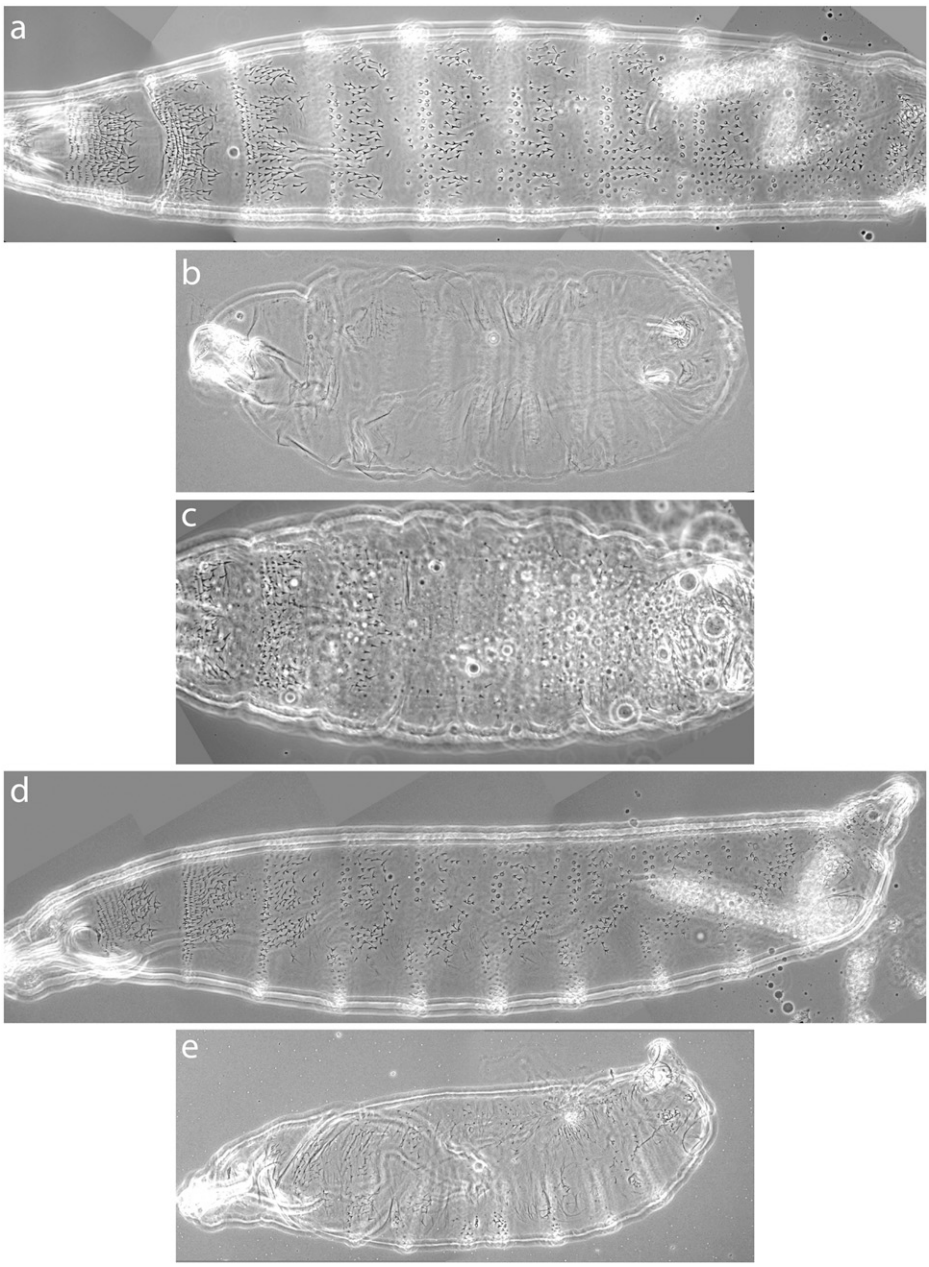

Fig. S4. Delivery of siRNA targeting the *svb* transcript in *D. virilis* embryos causes loss of dorsal and lateral trichomes in first-instar larvae. (*A*) Dorsal view of an embryo injected with DsiRNA targeting EGFP that displays a wild-type trichome pattern. (*B*) Dorsal view of an embryo injected with svb3 DsiRNA that displays loss of almost all dorsal trichomes. (*C*) Dorsal view of an embryo treated with svb3 DsiRNA via biolistics that displays loss of most dorsal trichomes. (*D*) Lateral view of an embryo treated with EGFP DsiRNA with biolistics that displays a wild-type trichome pattern. (*E*) Lateral view of an embryo treated with svb5 DsiRNA with biolistics displays loss of most lateral trichomes. In all panels, composite images were generated in Fiji (1) using the Pairwise stitching plugin (2).

1. Schindelin J, et al. (2012) Fiji: An open-source platform for biological-image analysis. *Nat Methods* 9(7):676–682.
2. Preibisch S, Saalfeld S, Tomancak P (2009) Globally optimal stitching of tiled 3D microscopic image acquisitions. *Bioinformatics* 25(11):1463–1465.

Table S1. Primers used to amplify enhancer constructs from *D. virilis*

Region name	Forward primer	Reverse primer
1	AGTGACCCTGGCCTAGGTCTAC	CACAGTAAACAAAACACTAATCCAAGTGCG
2longA	TGTGCAGCTAAAAAGCTACAAGATCC	CTTTGCTTGAACTTTGCTACGTTTCC
2longB	AGGAGTAGCAAGGTAGCGTTCTAAGG	CTGTGTGCCATAAATTGTCTTCAAGC
2A	TGCTTTCGGATTTGTTGTTG	TTCCATCTTTCCCTTGGTTG
3	GCGCGACCTAATCAAATTGCCAT	TGTGCGCTCTGATAATTGAACTCG
4	TGAGCAGATATTAACGTTTAAGAAGAGCG	AGTGCCAGTGACGTAATCACAGTAAC
5	TGAGGACAGAAGCGAAAGGT	TTGACTTTTGTTGCGTCTGC
6Ba	TGCTGCTGTAAGTGTTAAAGTTCG	GAGGAAAGATCCGATAGAATTGACC
6Bb	CTGCTGTTTTGTGTACGTGTTTGC	ACCAAGTTTAACTGAAGACCCACAGC
6A	TATGCTTGACGGCACTTCTG	ATTTCCCATAAGCAGCATGG
7	CCACCTCTTGTCAGCTGCACG	ACTTGAAGTTGCTCTACTAAGAGACAC
8	TACGTAACCGCCCAGAACTC	ATCTGCAGCCTGCTCATTCT
9Y	CGACTCTGGCATGGTTTAAGCTC	TGAGACAGAGTTCTGGGCGGTTA
9X2	GCCAACTATGTGAAATGGTTTCAATC	GTGTCCATACCCTTGATTTGAGGCC
9X1	AAAAGCTCAGGGCTTAACAAGAGC	GTTAACTAAACTTTATCCAAACGTGTCAGC
10	CCAATCGCATTCATTGTATCTGAATGTG	GAGCAACAATTGCGATATAAAGTTAATGTGC
11	CATGCAACAAGTCGCATATTGGTTGC	CTGAGTCAAAGGTTGATCAGAGTTGATC
Y	CCACTTCCACCTAGAACCGCTTT	GTGCAGCAAGGCAACCAATATG
X	AGAAGGCACTGAGAGAGGCGATT	AATATGCGTCCAGCTGCTCAGTC
15	GCGCTGTCTGCGCCATTCAC	AGCGGTGTATCCTTTGCAGCAAG
16	GAGCATGGAGTCAGAGAATATTAATTACG	GACTTAAACGTATTACAGGCAAGCGC
17	CTTGATCGCTGTGCTGAGCTGC	GCTTCACCGTTAAGCTAACTGCAC
18	CCCAAGTTCAATGTGAAACTGACG	AGTTCGGATTCTTTGAGTGCAACC
19	CCCAAACTGCTTTGTGATACC	GCAACTCTTTTTGCCAAGTGTG
20	CAGTTTCACAGCTCAAATGGATGG	TTCTGCTCAAGAGTTTGTGGATGC
21	AGAGAGAGCGAGTGTAGAATGAGAGG	CCAAAAGTTCAACTGACACAACTATCG
22	ATCAGCTGGACTTTCTTTTTGGTATCG	ACAGCACTTCCTCTCATTCTACACTCG
23	CAAAACCTGCAAAGCCATACGC	AATCCGTTCACAGTGATTAGCC
24	CCAGCATCGCATCATATTAGCC	TGGTGAGACGCTGACATAGCC
25A	GCTTTGACCACAAAGCTTAATAGGG	AGATGGATGGCTAGATGGTTAGTTGC
25B	CATATGACATGTTGCCAAGAAACG	GTCGCAGTCACAGCAGAGATATTAGC
26	ATTTTGCCCAAGGAAAAGAAGC	GCCCATTTTCAATAGCATCAGC
27A	AGCTCCTAGATGCGAACCTCAACC	TAGCACTTCAGACATCAAACAACTGC
27B	CTACCACAGGGGATAGATGAAAAAGC	TCATCGTTTAGGTGATTGTCTTACGG
28	GCGCAAATCACTTGCATATTCC	TCGGCTCTCTCCATCTCTCTCC

Table S2. Primers used to amplify enhancer constructs from *D. ezoana* and *D. littoralis*

Region name	Forward primer	Reverse primer
D. ezoana 3	CCGCGGCGACCTAATCAAATTGCCATTAAAACCG	GTCGACGCCAGCCAAAGCTTCCTGTTACTGTC
D. ezoana 8	CCGCGGACGATGGCGAAAGACGACCGCAACTG	GTCGACTTCTCTTCAGCCCCCTGCTTGTCAGCC
D. ezoana 19	CTCGAGTTTGACAGCCAGTTGAACGGACGCGG	CTCGAGTCCCCAGTAGTCCATTGCACTTGGGCG
D. littoralis 3	CCGCGGCGACCTAATCAAATTGCCATTAAAACCG	GTCGACGCCAGCCAAAGCTTCCTGTTACTGTC
D. littoralis 8	CCGCGGACGATGGCGAAAGACGACCGCAACTG	GTCGACGGCGTTGGCTTTGGCTTTGGCTTTGG
D. littoralis 19	CCGCGGTTTGACAGCCAGTTGAACGGACGCGG	GTCGACTCCCCAGTAGTCCATTGCACTTGGGCG

The underlined sequences correspond to restriction enzyme sites used for cloning.