

Table S1. Probes and primers

Name	Sequence	Binding	Position (nt)
Zelda gel-shift probe sequences for testing a binding site in the eve 3+7 enhancer (Fig. 2A)*			
Wild type	AACGCTCTAC TTACCTG CAATT	Yes	403-424
Mutant	AACGCTC ACTAGTAG TGCAATT	No	
Primers for PCR amplification of eve 2 sequences (Fig. 3F)†			
Fragment 1			
5' oligo	GGCAGGAGCGAGGTATCCTTCTG	Yes	-23-129
3' oligo	CTGCAATTGACTAATAATCTCGCTG		
Fragment 2			
5' oligo	GTTAATCCGTTTGCCATCAGCGAG	Yes	88-250
3' oligo	CCCTCGATTCCGTCTAAATGAAAG		
Fragment 3			
5' oligo	TTTAGACGGAATCGAGGGACCCTG	Yes	233-382
3' oligo	GGATTCCAAGTCAAGCCCTTGGC		
Fragment 4			
5' oligo	GCCAAGGGCTTGACTTGGAATCC	Yes	360-513
3' oligo	CCGTTAATTGCGTTGCCTGGACC		
Probe sequences for testing Zelda binding to the eve 2 enhancer (Fig. 3G,H)‡			
P32	TAGCCAAGGGCTTGACTTGGA	No	358-378
P33	CTTGGAATCCAATCCCGATCC	No	373-393
P34	CGATCCCTAGCCCGATCCCAA	No	388-408
P35	TCCAATCCAATCCAATCC	No	403-423
P36	CAATCCCTTGCCTTTTTCATT	No	418-438
P37	TTCATTAGAAAGTCATAAAAA	No	433-453
P38	TAAAAACACATAATAATGATG	No	448-468
P39	ATGATGTGCAAGGGATTAGGG	No	463-483
P40	TTAGGGGCGCGCAGTCCAGG	No	478-498
P41	TC CAGGCAA CGCAATTAACGG	Yes	493-513
P42	GCGCAGGTCC CAGGCAA CGCAAT	Yes	486-507
P43	GCGCAGGAGC GAGGTAT CCTTCC	Yes	-23 to -2
P44	CTTCTGGTTAC CCGGTACT GCAT	Weak	-6-18
P45	CGAAAAGCTGGC CTGGTTI CTCGC	Yes	52-75
P46	CGTGTTAATCCGTTTGCCATCA	No	85-106
P47	GTGCGAGTTTGGTAACACGCTG	No	198-219
P48	CTTTCATTTAGACGGAATCGAGGG	No	227-250
P49	CAGGGCATTCCGCCGATCTAGC	No	292-313
Knirps gel-shift probe sequences for testing binding sites in the eve 3+7 enhancer (Fig. 4A, Fig. 5)§			
P1	GACACAAGGATCCTCGAAATC	No	-7-14
P2	CTCGAA ATCGAGAGC ACCTC	Yes	6-26
P2 M1	CTCGAA GTCGATAGAG ACCTC	No	
P3	TGCATTAGAA AACTAGATCAG	Yes	29-49
P3 M1	TGCATTAG CTAGCTAGATCAG	No	
P3 M2	TGCATTAGAA GACTAAATTAG	No	
P3 M3	TGCATTAGAA AACTAAATTAG	Weak	
P3 M4	TGCATTAGAA AACTAAATCAG	Yes	
P3 M5	TGCATTAGAA AACTAGATTAG	Yes	
P4	TCAGTTTTTTGTTTTGGC CGACCGATTT TTGTG	Yes	46-78
P4 M1	TCAGTTTTTTGTTTTGGC CTACAGATT CTTGTG	Yes	
P4 M2	TCAGTTTTTTGTTTT AGCTTACAGATT TTTGTG	Yes	
P5	GTGCCCGG TGCTCTCTT ACG	Yes	76-96
P5 M1	GTGCCCGGT ACTATCTT CACG	No	
P5 M2	GTGCCCGGT ACTATCTT ACG	No	
P6	TATGGCCG CGTCCCAT TCC	Yes	100-120
P6 M1	TATGGCCG CCTAGCCAT TCC	No	
P6 M2	TATGGCCG CATTACCAT TCC	No	
P7	AGCTTCTTTGTTCCGGGCTCA	No	122-142
P8	CAGAAATCTGTATGGAATTATG	No	141-162
P9	TTATGGTATATGCAGATTTTATGG	No	158-182
P10	TTATGGGTCCCGGCGAGTCCGGTTC	No	177-200
P11	GGTTCGCGGAACGGGAGTGTC	No	196-216
P12	GTGTCCTGCCGCGAGAGTCTCCTCGC	No	212-236
P13	CTCGCCGGCGATCCTTGTGCGC	No	232-252
P14	TTGT CGCCGAT TAGGA	Weak	246-263
P14 M1	TTGT CA CCAGTATTAGGA	No	
P15	CGTATTAGGA AAAGTAGATCAC	Yes	254-274
P15 M1	CGTATTAG CTAGGTAGATCAC	No	
P15 M2	CGTATTAGGA GAGTATATTAC	No	

P16	GTAGATCAGTTTTTTGTTC	No	266-285
P17	ACGTTTTTTGTTCCATTGTG	No	273-293
P18	ATTGTGCGCTTTTTTCGCT	No	288-306
P19	TTTTTCGCT <u>IGCGCTAGTTTTT</u>	Yes	298-318
P19 M1	TTTTTCGCT <u>ACGATAGTCTTT</u>	No	
P19 M2	TTTTTCGCT <u>ACGATAGTTTTT</u>	No	
P20	GCTAGTTTTTTTCCCCGAACC	No	309-329
P21	GAACCCAGCGAACT <u>TGCTCTAATT</u>	Yes	325-347
P21 M1	GAACCCAGCGAACT <u>ACTATAATC</u>	No	
P21 M2	GAACCCAGCGAACT <u>ACTATAATT</u>	No	
P22	TAATTTTTTAATTCTTCACGGCTTTT	Weak	343-368
P23	TTTTATTGGGCTCCTGGAAAAACG	Weak	365-389
P24	AAACGCGGACAAGTTATAACGC	No	385-407
P25	AA <u>CGCTCTACTT</u> ACCTGCAATT	Yes	403-424
P25 M1	AA <u>CACTTACTT</u> ACCTGCAATT	No	
P25 M2	AA <u>CGCCGGTCTT</u> ACCTGCAATT	No	
P26	AATTGTGGCCATAACTCGCACTGC	No	421-444
P27	ACT <u>TGCTCTCGTTTTT</u> AAGAT	Yes	440-459
P27 M1	ACT <u>TGCTCTCCTAGTT</u> AAGAT	No	
P27 M2	ACT <u>ACTATCGTCTTT</u> AAGAT	No	
P27 M3	ACT <u>ACTATCGTTTTT</u> AAGAT	No	
P28	GTTTTTAAGATCCGTTTGT	No	449-467
P29	TTTGTGGTGGTTGTT <u>IGTCCGCGAT</u> GGCAT	Yes	463-493
P29 M1	TTTGTGGTGGTTGTT <u>ATCAGCGAC</u> GGCAT	Yes	
P30	GGCATTACGTTTTTACGAGC	No	489-509
P31	ACGAGCTCGTTCCTTCGGGTCCA	No	504-526

*The TAGteam sequence is underlined. Numbers refer to nucleotide position with respect to the minimal eve 3+7 enhancer tested in vivo and reported in Fig. S4.

[†]Numbers refer to the nucleotide position with respect to the minimal eve 2 enhancer tested in vivo, which spans from the *Bst*II site on the 5' side to the *Bss*III site on the 3' side (i.e. nucleotides 1-489).

[‡]The putative Zelda binding sites are underlined. Numbers are as above.

[§]Knirps binding sites are in blue. Tested mutations are in red. Numbers refer to the nucleotide position with respect to the minimal eve 3+7 enhancer tested in vivo and reported in Fig. S4.