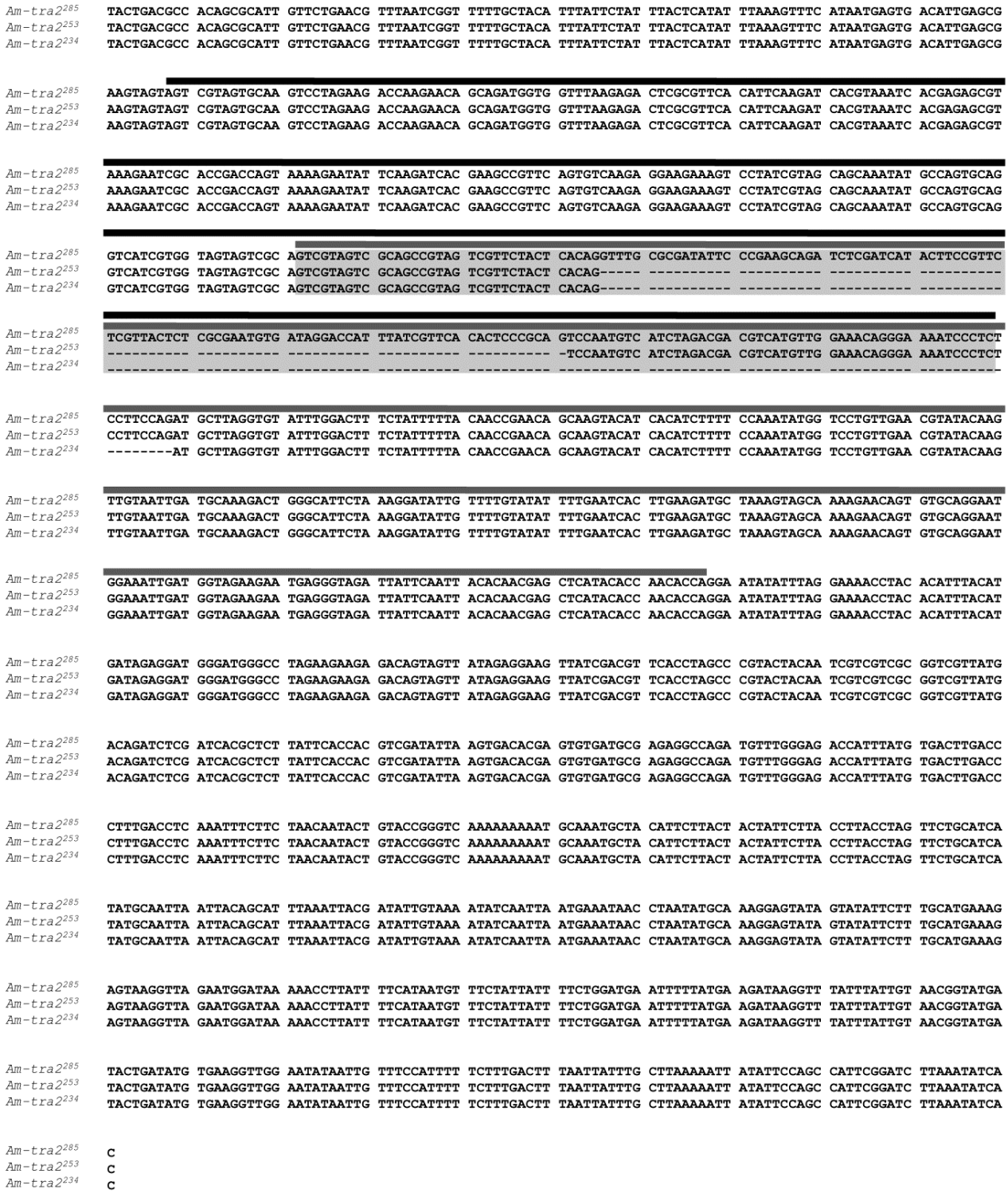

Figure S1 Multiple Nucleotide Sequence Alignment of the Am-tra2 cDNAs *Am-tra2*^{285}, *Am-tra2*^{253}, *Am-tra2*^{234}. Black (dsRNA-2) and grey (dsRNA-1) boxes mark the region that we used to produce our dsRNAs.

Figure S2 Multiple Sequence Alignment of the Tra2 proteins of *Apis mellifera (A.m.), Drosophila melanogaster (D.m.)* and *Bombyx mori (B.m)*. The RNA binding domain (RBD) is highlighted in black, and the linker region is highlighted in dark grey. The arginine-serine rich motifs (RS1 and RS2) are shown in light grey. The RNP-1 and RNP-2 sequence elements are marked by black bars.

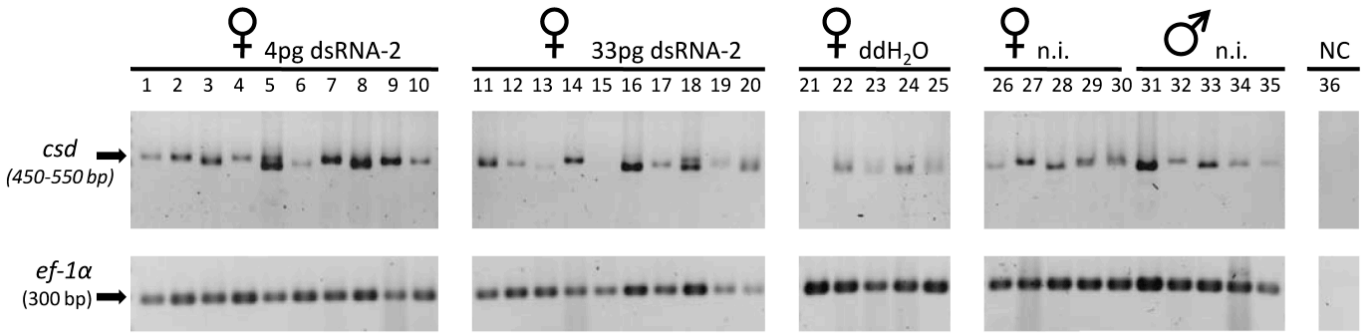

Figure S3 Constitutive splicing of *csd* transcripts in *Am-tra2* dsRNA-2 treated embryos. The *csd* mRNAs of individuals 77-80 hours after egg-laying were studied using semiquantitative RT-PCR. Early embryos were injected with 4 pg of *Am-tra2* dsRNA-2 (lanes 1-10), 33 pg of *Am-tra*2 dsRNA-2 (lanes 11-20) or ddH_20 (lanes 21-25). The untreated female and male controls (labeled as n.i.) are shown in lanes 26-30 and 31-35, respectively. NC denotes our control PCR in which no cDNA was added (lane 36). Fragments corresponding to the *csd* transcripts including the hypervariable region (size of ~450-550 bp) were resolved by agarose gel electrophoresis and stained with ethidium bromide. The size of *csd* fragments varies due to length differences of the hypervariable that substantially varies between *csd* alleles. Because *csd* alleles can vary substantially in the nucleotide sequence (10-15%) we cannot amplify to the same extend all the *csd* alleles. We amplified cDNAs of the gene *elongation factor 1α* (ef-1α) as a relative control to semiquantify *csd* transcripts across embryonic samples.

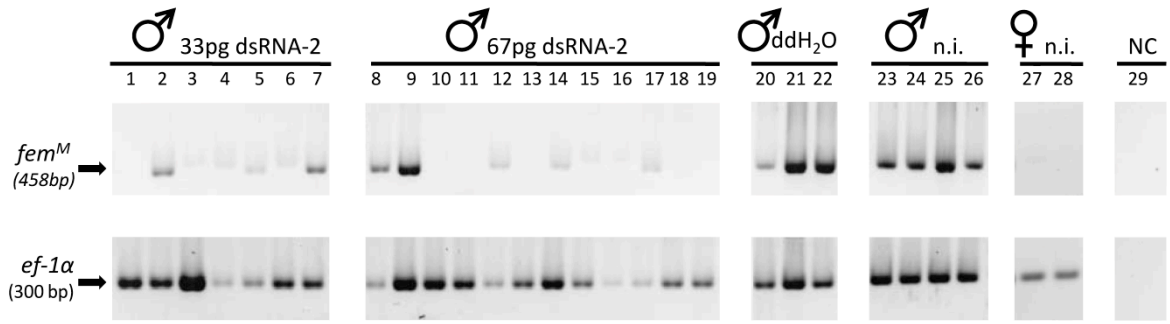

Figure S4 *fem* transcripts in male embryos treated with *Am-tra2* dsRNA-2. The male *fem* mRNAs of individuals 48 hours after egg-laying were studied using semiquantitative RT-PCR. Early embryos were injected with 33 pg of *Am-tra2* dsRNA-2 (lanes 1-7), 67 pg of *Am-tra*2 dsRNA-2 (lanes 8-19) or ddH_20 (lanes 20-22). The untreated male and female controls (labeled as n.i.) are shown in lanes 23-26 and 27-28, respectively. NC denotes our control PCR in which no cDNA was added (lane 29). Fragments were resolved by agarose gel electrophoresis and stained with ethidium bromide. The corresponding fragment of the male *fem* transcripts has a size of 458 bp. We used amplification of the cDNAs of the gene *elongation factor 1α* (ef-1α) as a relative control to semiquantify *csd* transcripts across embryonic samples.

Table S1 Sequences of oligonucleotides that were used

to synthesize dsRNA	
#22M: tra-2_ds_FOR	TAATACGACTCACTATAGGGCGAAGTCGTAGTCGCAGCCGTAGTCGTT
#23M: tra-2_ds_REV	TAATACGACTCACTATAGGGCGACTGGTGTTGGTGTATGAGCTCGTTG
#591	TAATACGACTCACTATAGGGAGTCGTAGTGCAAGTCCTAGAAGACC
#592	TAATACGACTCACTATAGGGATTTTCCCTGTTTCCAACATGAC
to analyze *Am-dsx* splicing	
#417	CTATTGGAGCACAGTAGCAAACTTG
#418	GGCTACGTATGTTTAGGAGGACC
#419	GAAACAATTTTGTTCAAAATAGAATTCC
to analyze *fem* splicing	
#412	CTGATTTTTCAATATTTACAGCTAAAACTGTAC
#523	CAACATCTGATGAACTTAAACGG
#410	TGAAGTTAATAACATATTTTTAATTCATCAATGAAG
#566	TGTACCATCTGAAGATTCTAATTTTTCG
to amplify *elongation factor-1α*	
#EM033	CGTTCGTACCGATCTCCGGATG
#EM034	GCTGCTGGAGCGAATGTTAC
in 5'RACE experiments	
5'RACE OLLI:	TGAACGGCTTCGTG
in 3'RACE experiments	
#33M (3'RACE J1 OUTER)	ACT CTC GCG AAT GTG ATA GGA CCA T
#34M (3'RACE J2 INNER)	TCA CAC TCC CGC AGT CCA ATG TCA T
3'RACE OLLI	AGAACAGTGTGCAG
to clone full ORF of *Am-tra2*	
#359	GATCGGATCCATGAGTGACATTGAGCGAAGTAGTAG
#421	TGACACGCGTTTAATATCGACGTGGTGAATAAGAGC
to amplify *csd* transcripts	
#CS-1	ATGAAAAAGAAAAACTTTTAGAAGAAAGAAC
#CS-2	TAAAATTTTTATAGTTTTCATTGATGCGTAG