



**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-tra2* dsRNA-2 (lanes 11-20) or ddH<sub>2</sub>O (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.