SUPPLEMENTARY DATA

Figure Legends

Figure S1. Schematic diagram of alternative splicing in *dsx* and *tra2* pre-mRNAs. The exon-intron organization and alternative splicing of the *dsx* and *tra2* genes are depicted. Splicing patterns observed in the presence and absence of bound Tra2 are indicated. The position of the ISS within the M1 intron is indicated by the thick line. Arrows indicate the position of the somatic and germline promoters. The sequence of the ISS and the positions of CAAGR repeats (underlined) within it is shown. The expanded parts of female-specific exon 4 illustrate the distribution of individual *dsx* ESE elements and their sequences. Introns are not drawn to scale.

Figure S2. Gel shift analysis of MCP and MCP-Tra2 binding to splicing substrates. Radiolabeled ftzMS2-10 (intact binding sites) and ftzMS2mt (mutant binding sites) RNA substrates were incubated with either MCP or MCP-Tra2 as indicated above the lanes. RNA-protein complexes were resolved on a 3.5% non-denaturing poly-acrylamide gel. The positions of free RNA substrates and RNA-protein complexes are indicated.

Figure S3. Position dependent Tra2 function in human nuclear extracts
In vitro splicing reactions with substrates ftzMS2-10 and ftz-MS2E were carried out in Hela nuclear extracts supplemented with 100-400 nM MCP or MCP-Tra2.

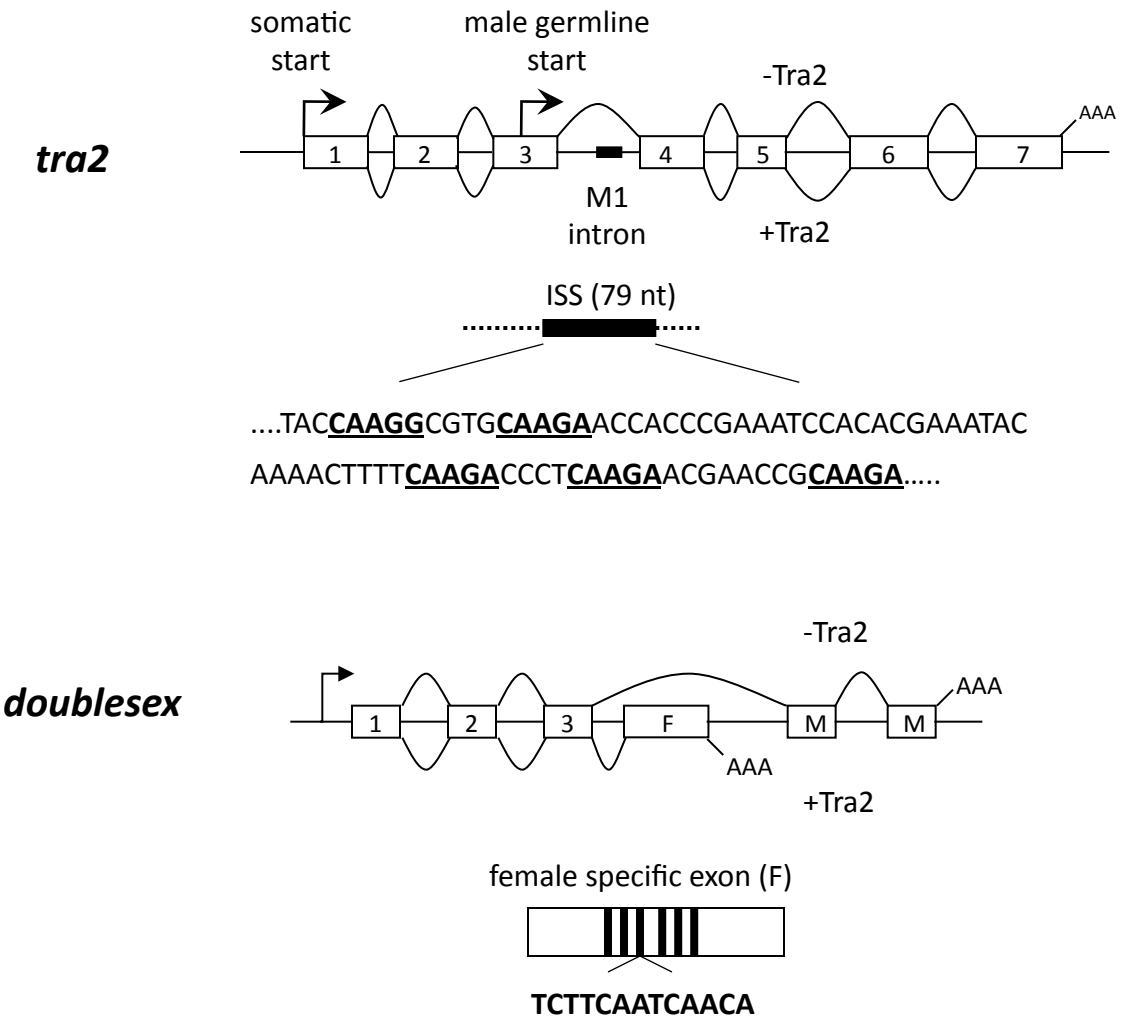
tra2
somatic start
male germline start
-Tra2
AAA
1
2
3
4
5
6
7
M1 intron
+Tra2
ISS (79 nt)
....TACCAAGGCGTGCAAGAACCACCCGAAATCCACACGAAATAC
AAAACTTTTCAAGACCCTCAAGAACGAACCGCAAGA.....
doublesex
-Tra2
AAA
1
2
3
F
M
M
AAA
+Tra2
female specific exon (F)
TCTTCAATCAACA

Figure S1

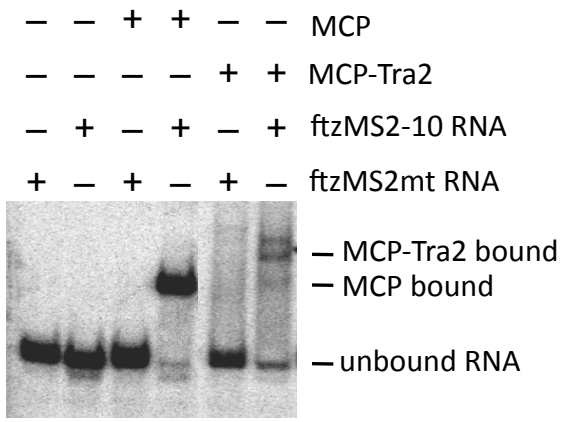
– – + + – – MCP
– – – – + + MCP-Tra2
– + – + – + ftzMS2-10 RNA
+ – + – + – ftzMS2mt RNA
– MCP-Tra2 bound
– MCP bound
– unbound RNA

Figure S2

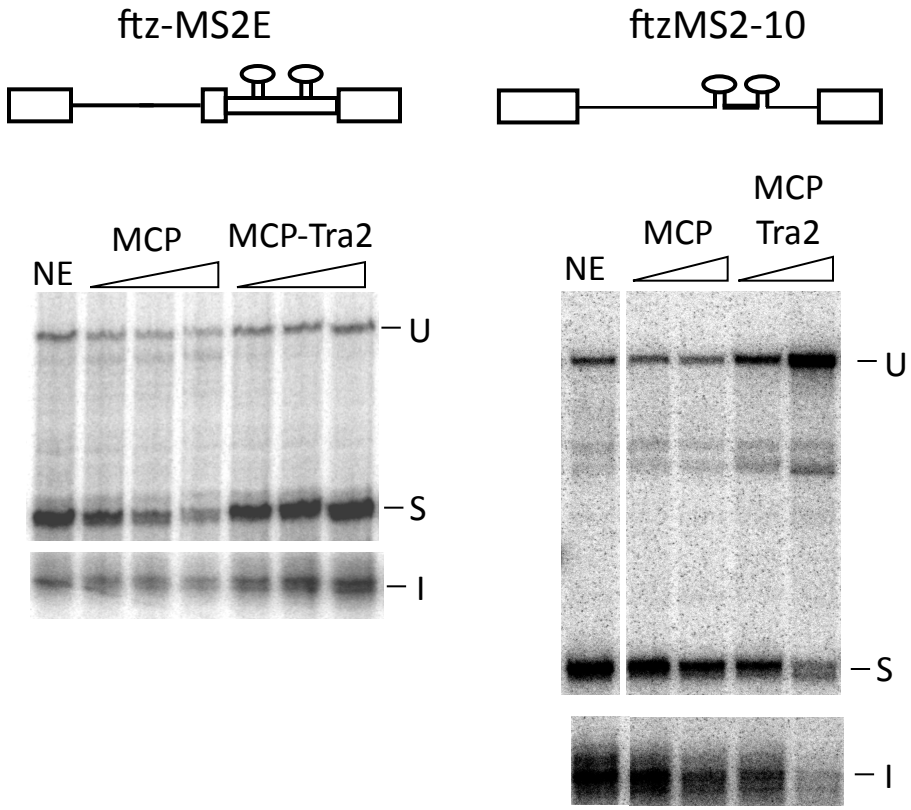

Figure S3