

Supplementary Materials

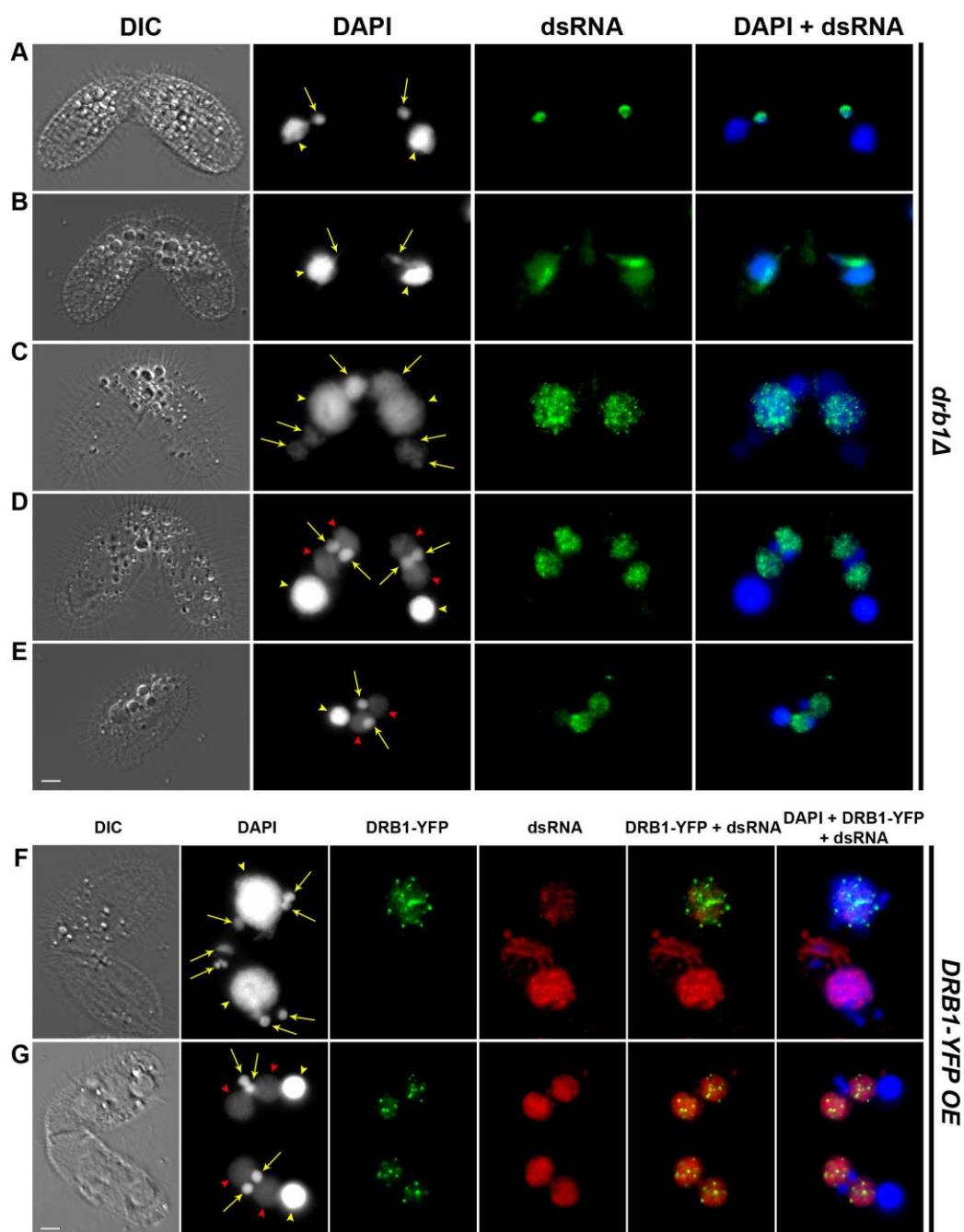


Fig. S1

Nuclear localization of dsRNAs in mating cells that are deficient or overexpressing *DRB1*

Staining pattern of dsRNAs in the following stages of *drb1Δ* mating are examined: (A) pre-meiosis; (B) meiotic prophase, crescent formation; (C) completion of meiosis and

cross-fertilization; (D) new MAC development; (E) pair separation. Two stages of mating cells overexpressing (OE) *DRB1-YFP* with specific Drb1p localization patterns were examined: (F) completion of meiosis and cross-fertilization; (G) new MAC development. (A-E) Immunostaining of dsRNA-specific mouse monoclonal antibody J2 was labeled with Alexa Fluor® 488-conjugated goat anti-mouse IgG (colored green in the right two columns). (F, G) The secondary antibody labeling for dsRNA is Rhodamine Red™-X (RRX) AffiniPure Donkey Anti-Mouse IgG (colored red in the right three columns) due to yellow fluorescent protein (YFP) tagging (colored green in the third and right two columns). Cells in conjugation are shown as reference in DIC. DNA was counterstained with DAPI (the second column, colored blue in the right column). Yellow arrows indicate MICs, yellow arrowheads, parental MACs and red arrowheads, new MACs. Bar, 5 μm . All pictures were taken with the same magnification.

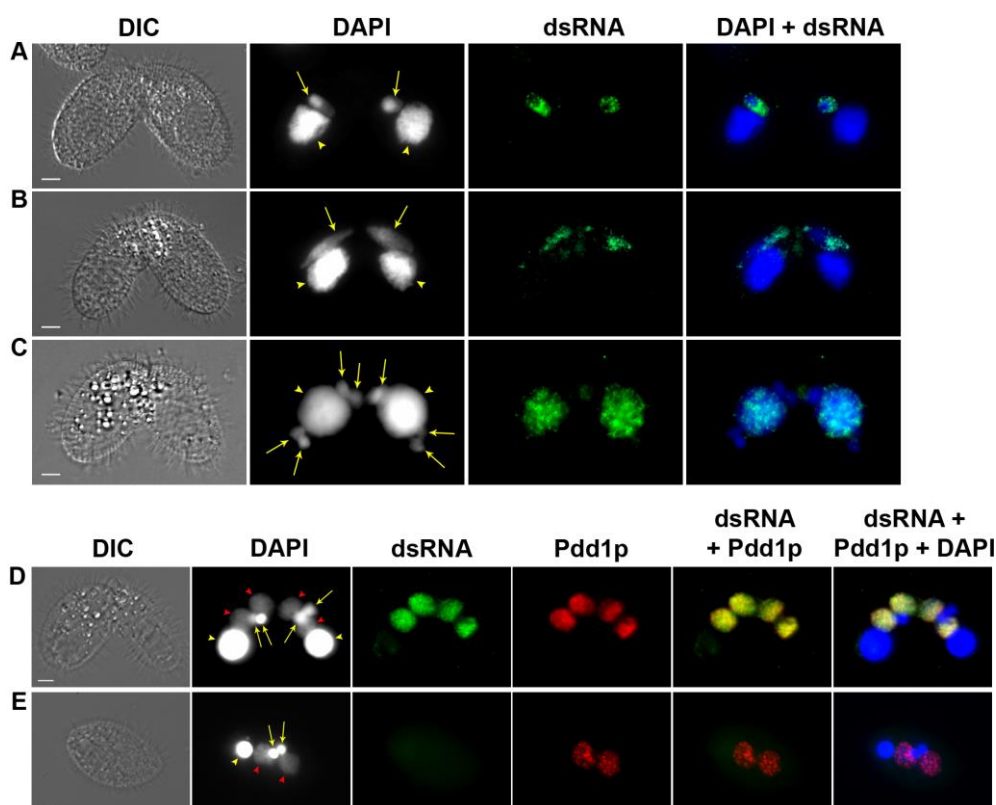


Fig. S2

Nuclear localization of dsRNAs in mating cells that are deficient in *DRB2* (germline knockout, GKO).

Staining patterns of dsRNAs in the following stages of *drb2Δ* GKO mating were examined: (A) pre-meiosis; (B) meiotic prophase, crescent formation; (C) completion of meiosis and cross-fertilization; (D) new MAC development; (E) pair separation. (A-D) Immunostaining of dsRNA-specific mouse monoclonal antibody J2 was labeled with Alexa Fluor®488-conjugated goat anti-mouse IgG (colored green). (D, E) Staining of Pdd1p was included in late stages to examine the effect of *drb2Δ*. Alexa Fluor®568-conjugated goat anti-rabbit IgG was used as the secondary antibody for Pdd1p immunostaining (colored red in the right three columns). Cells in conjugation are shown as reference in DIC. DNA was counterstained with DAPI (the second column, colored blue in the right column). Yellow arrows indicate MICs, yellow arrowheads, parental MACs and red arrowheads, new MACs. Bar, 5 μm. Pictures were taken with the same magnification as in panel A to C and D to E.

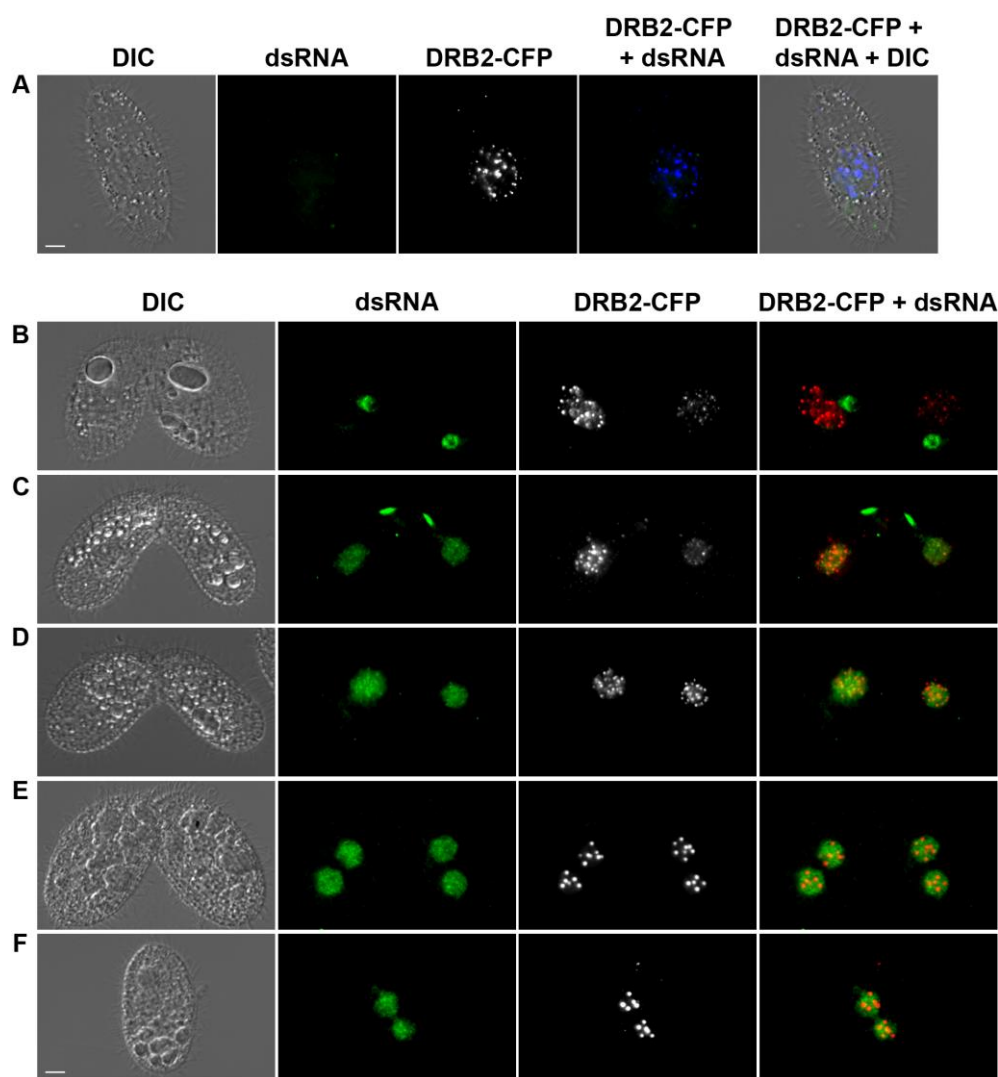


Fig. S3

Staining of dsRNAs is not affected in cells that overexpress *DRB2-CFP*.

(A) *DRB2-CFP* was induced for expression by addition of CdCl_2 into starvation cultures for two-hour incubation. Unlike the hair-pin RNA-expressing cells, overexpression of the protein-coding gene *DRB2* (colored blue in the right two columns) from the same vector did not induce staining of dsRNAs (colored green in the second and right two columns). The effect of *Drb2p* overexpression on dsRNA staining during mating was examined in the following stages: (B) pre-meiosis; (C) crescent formation; (D) late meiosis or cross fertilization, not indicated by DAPI-staining due to the use of cyan fluorescence; (E) new MAC development; (F) pair separation. (B-F) dsRNA is colored green in the second and right column; *DRB2-CFP* is colored red in the right column for the analysis of colocalization. (A-F) dsRNA staining was labeled by Alexa Fluor®488-conjugated goat anti-mouse IgG and the signals were captured through YFP channel to avoid bleed-through of CFP signal in FITC channels.

Table S1. List of DNA oligomers

Primer name	5' to 3' Sequence
<u>PmeI</u> -GFP-F1	CGTTTAAACAGTAAAGGAGAAGAACTTTT
<u>XmaI</u> -GFP-R1	ATGTCCCGGGGACAGGTAATGGTTGTCTG
<u>ApaI</u> -GFP-F2	CGGGCCCAGTAAAGGAGAAGAACTTTT
<u>XhoI</u> -GFP-R2	GCTCGAGGGACAGGTAATGGTTGTCTG
M-plus-RT	AAATAAGACTAATCTATAAATAAGG
M-minus-RT	CTTATCAGTTAGATTGTTTGAAACTG
M-5'-2new	AGTTGTTTATTCTAAAATTTATCC
M-3'-2	GGAGAAGGATTCAACAAAGTAAGC
M-IES-R3	AATTGTGATTAATGCAAATACTATTCG
M-IES-F4	AGCATTACAACCTTGATGAGAACTG
R-element (R)	GATTTACTGTAAGATAGTTCTAG
R-minus-RT-1	AAATAAAATGAAATCTTAAGTTAGAATAG
R-3'-2	TAAGATAGTTCTAGAATAAGAC
R-IES-1F	TTAAACAGTGTA AAAACCCAA

* Restriction site sequence underlined.