

Expanded View Figures

Figure EV1. Knockdown effects of ISWI paralogs in *Paramecium tetraurelia*.

A, B Northern blot analysis using ISWI1-specific and ISWI5-specific probes, respectively. rRNA probe was used as a loading control against ribosomal RNA. Early: ~50% of cells with fragmented parental macronucleus; Late: the majority of cells with a visible anlagen. *ND7*-KD is used as a control to confirm mRNA expression.

C–F (C and E) Survival test graph. Dead cells are represented in red, sick in orange and cells diving at a normal rate in green. *PGM*-KD is used as a positive control, and *ND7*-KD as a negative control. (D and F) IES retention PCR (cropped inverted images). Five maternally controlled IES and five non-maternally controlled IESs are shown. The IES+ band represents retained IES; the IES- band represents excised IES; additional bands are likely PCR artifacts or primer dimers.

Source data are available online for this figure.

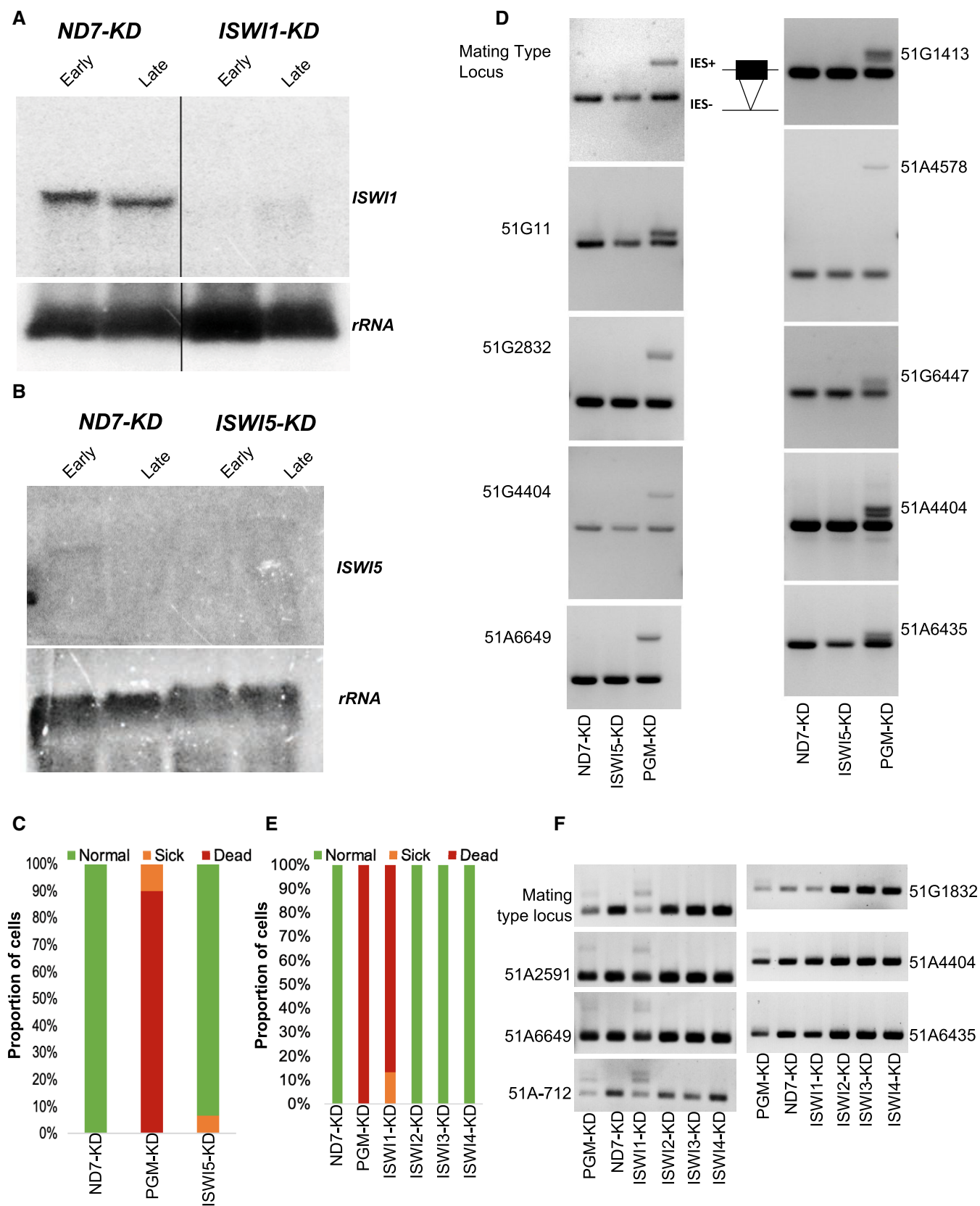


Figure EV1.

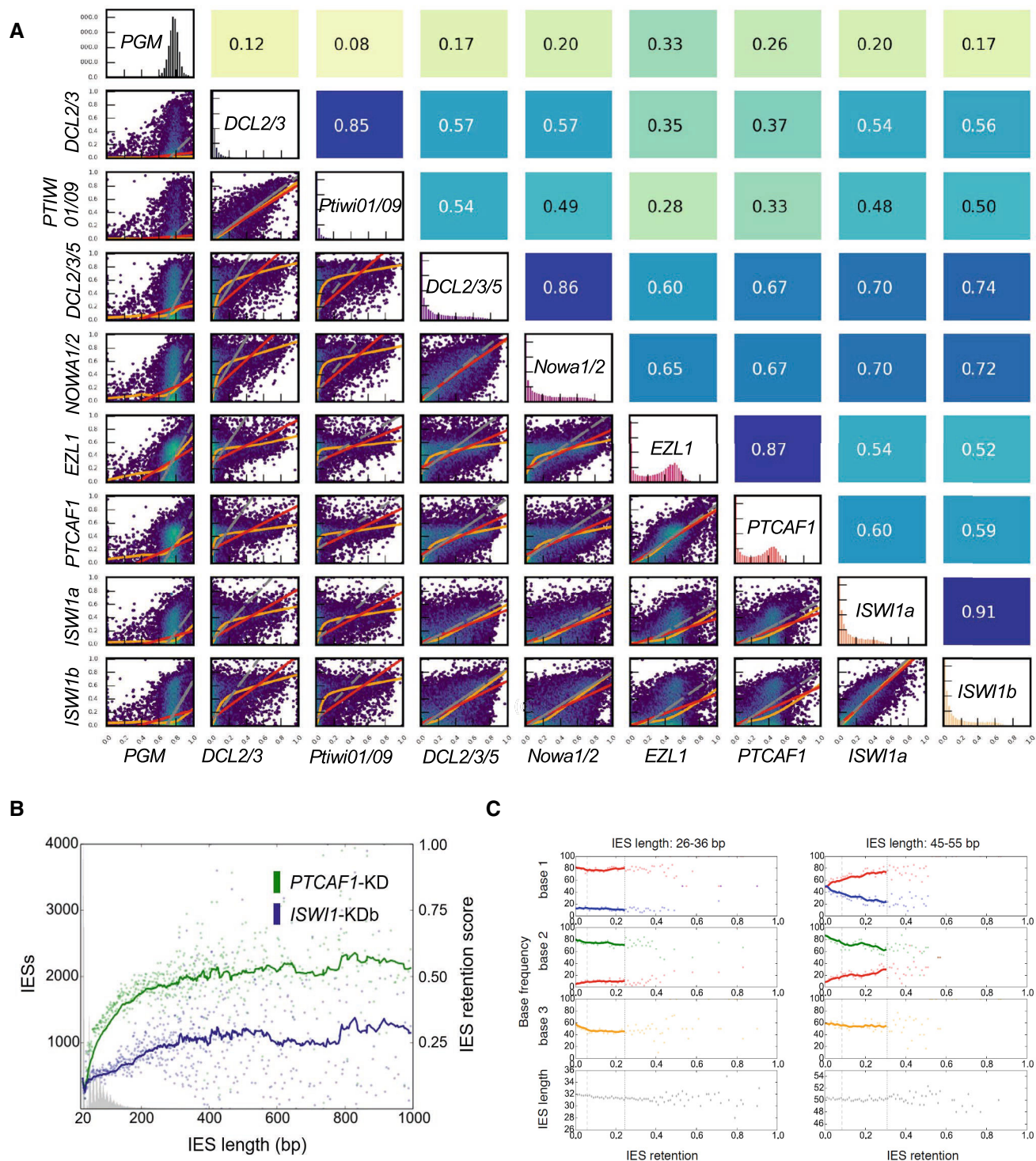


Figure EV2. Relationship between IES retention scores, IES length, and base sequences.

A Relationships in IES retention among knockdown pairs. Hexagonal binning of IES retention scores was used to generate the plots. Pearson's correlation coefficients are given above each subgraph. Red lines are for ordinary least-squares (OLS) regression, orange lines are for LOWESS, and gray lines are for orthogonal distance regression (ODR).

B IRSs versus IES length as described previously.

C Base frequencies of the first three bases after the TA repeat relative to the IRS of *ISWI1-KDb* from the first and third *Paramecium tetraurelia* IES length peak.

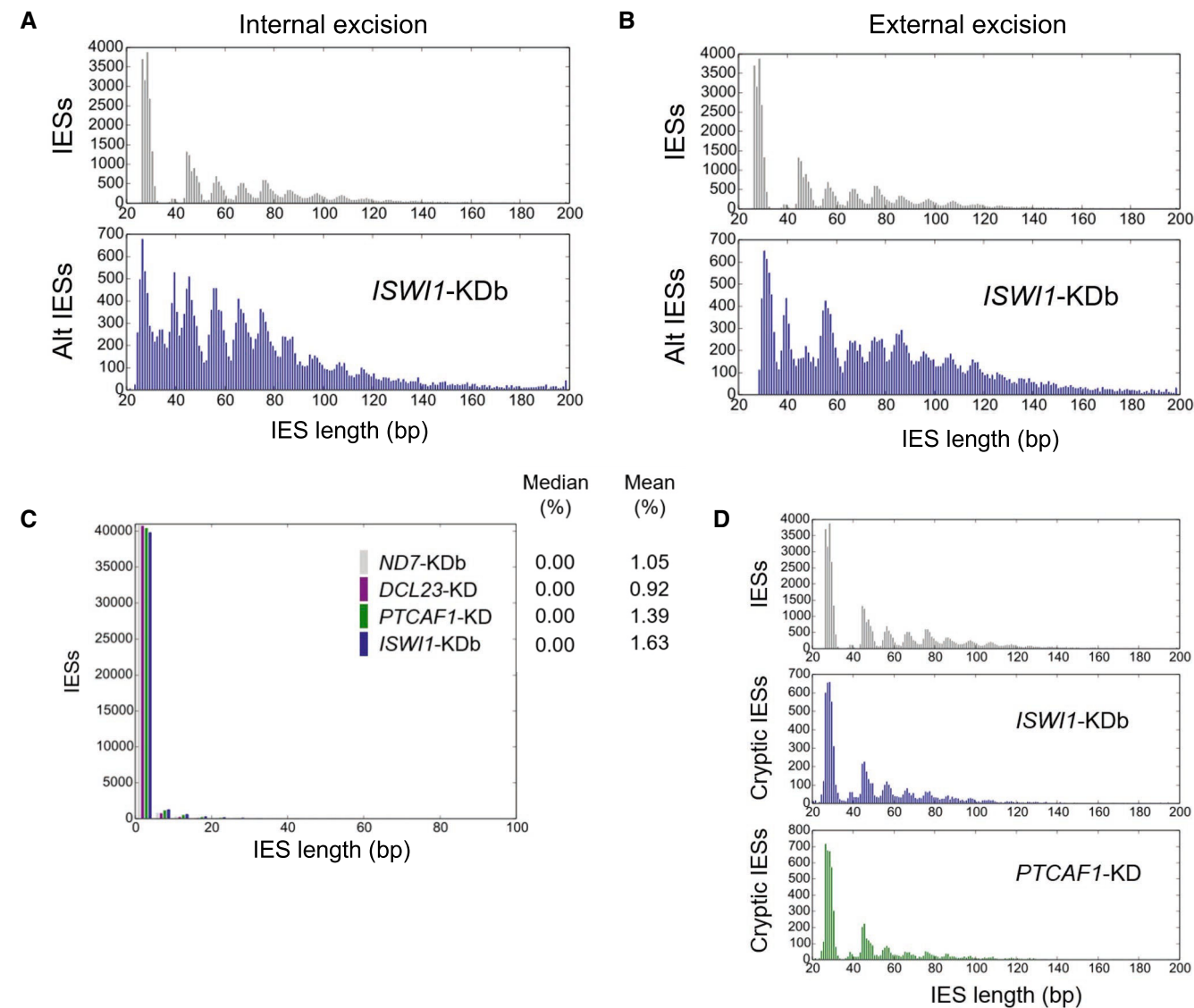


Figure EV3. Size distribution of Alternatively excised IESs and Cryptic IESs.

A Length distribution of internally excised alternative (Alt) IES boundaries.

B Length distribution of externally alternative (Alt) excised IES boundaries, respectively.

C Genome-wide analysis of cryptic IES excision. Cryptic excision (%) = $100 \times (\text{cryptically excised reads}) / (\text{all reads})$.

D Length distribution of cryptically excised IES.

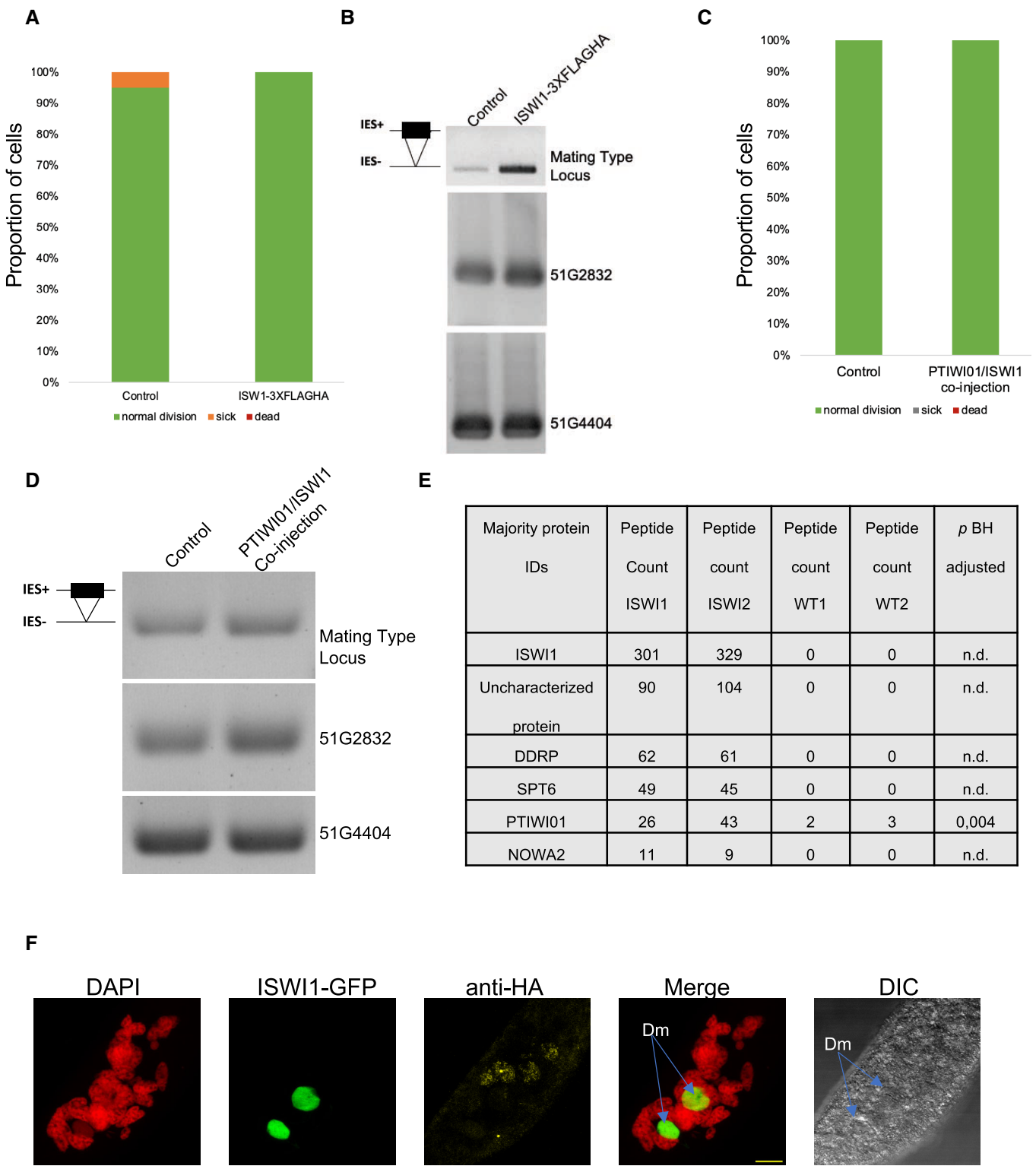


Figure EV4.

Figure EV4. Overexpression of fusion proteins do not show any adverse effects.

- A–D (A and C) Survival test graph. Dead cells are represented in red, sick in orange and cells diving at a normal rate in green. (B and D) IES retention PCR (cropped inverted images). Mating type, 51G2832, and 51G4404 IESs are shown. The IES+ band represents retained IES; the IES–band represents excised IES; additional bands are likely PCR artifacts or primer dimers.
- E Most abundant proteins in the ISWI1-3XFLAGHA MS analysis ISWI1 & ISWI2 are two biological replicates for ISWI1-3XFLAGHA, while WT1 and WT2 are biological replicates for control in MS analysis. Peptide count refers to the number of peptides detected in MS. Adjusted *P*-values were calculated following the Benjamini and Hochberg correction for multiple testing.
- F ISWI1-GFP localization to developing macronucleus seen in green during development; merge is an overlay of DAPI (red) staining parental and developing macronucleus, ISWI1-GFP (green), and anti-HA (yellow); scale bar = 10 μ m.

Source data are available online for this figure.

Figure EV5. Nucleosome density measurements after DNase-seq.

- A IES Retention Score (IRS) distributions for *PGM/ND7*-KD and *PGM/ISWI1*-KD. (B, C) Histograms of outer paired-end distances of mapped DNase-seq reads.
- D–I Normalized nucleosome density histograms for IESs weakly ($IRS < 0.2$) or more strongly retained in *ISWI1*-Kdb ($IRS \geq 0.2$), either for *ND7/PGM*-KD or *NOWA1/PGM*-KD. Kolmogorov–Smirnov statistics and their *P*-values are provided. Titles for graphs give criteria for IES selection.

Source data are available online for this figure.

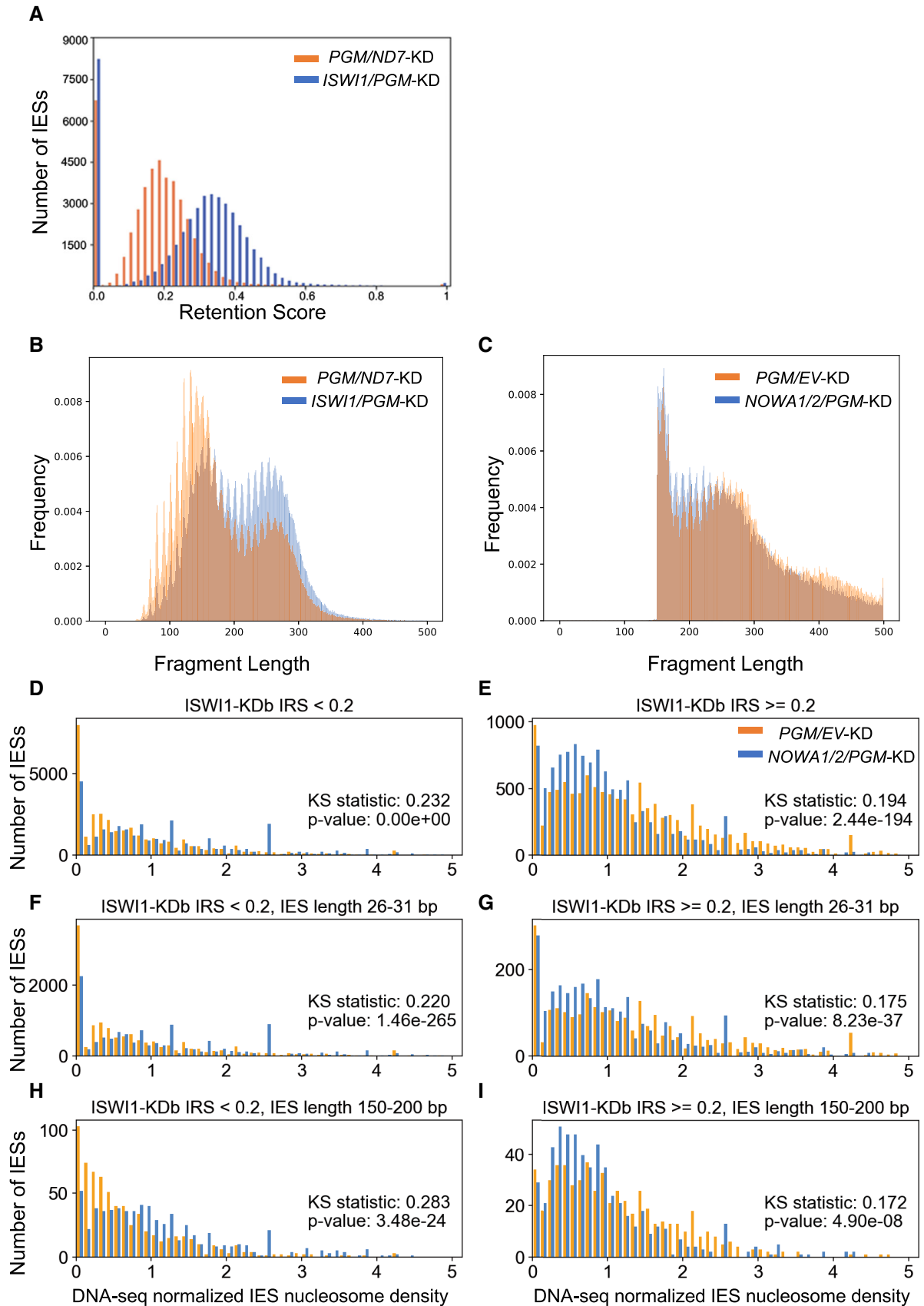


Figure EV5.