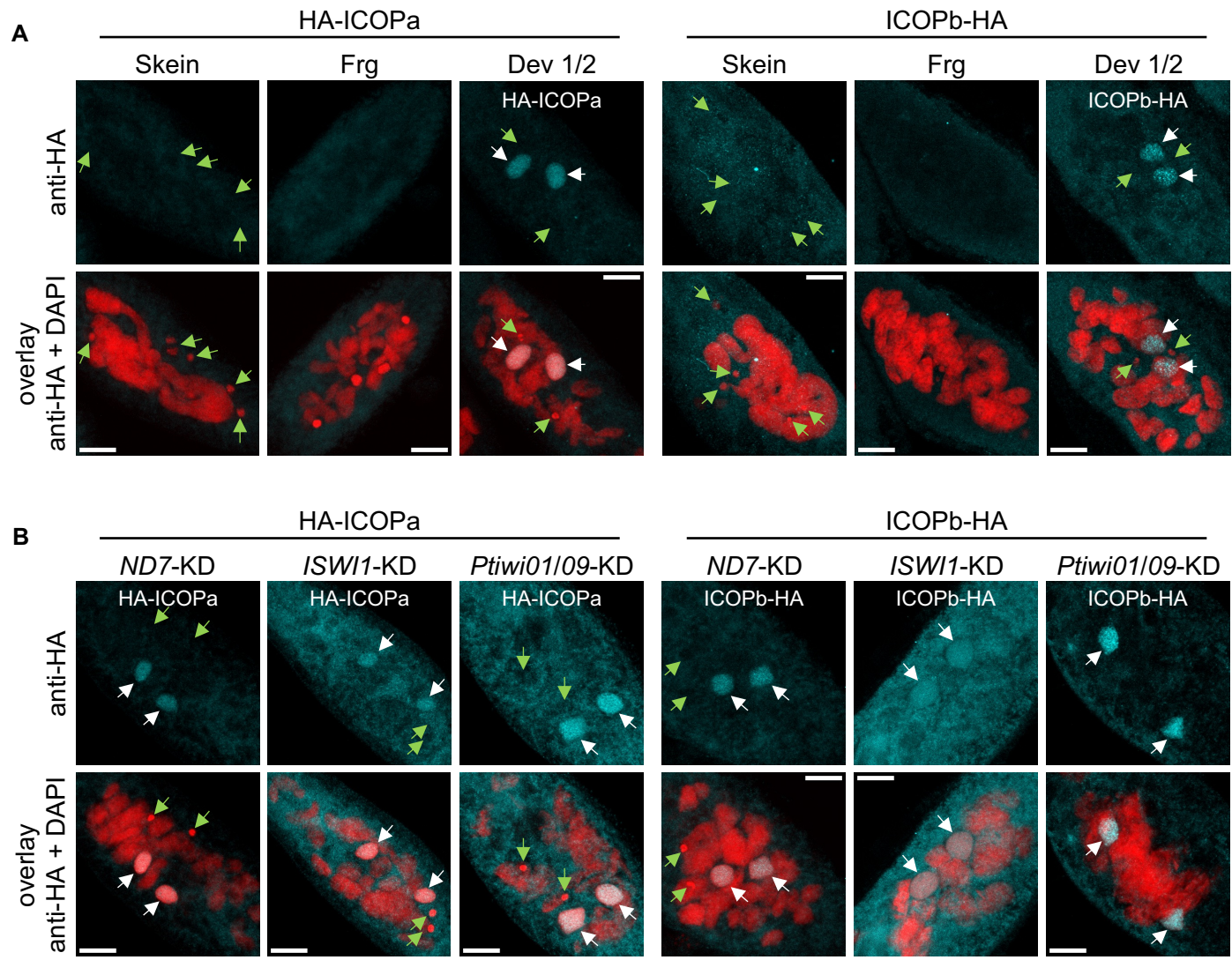


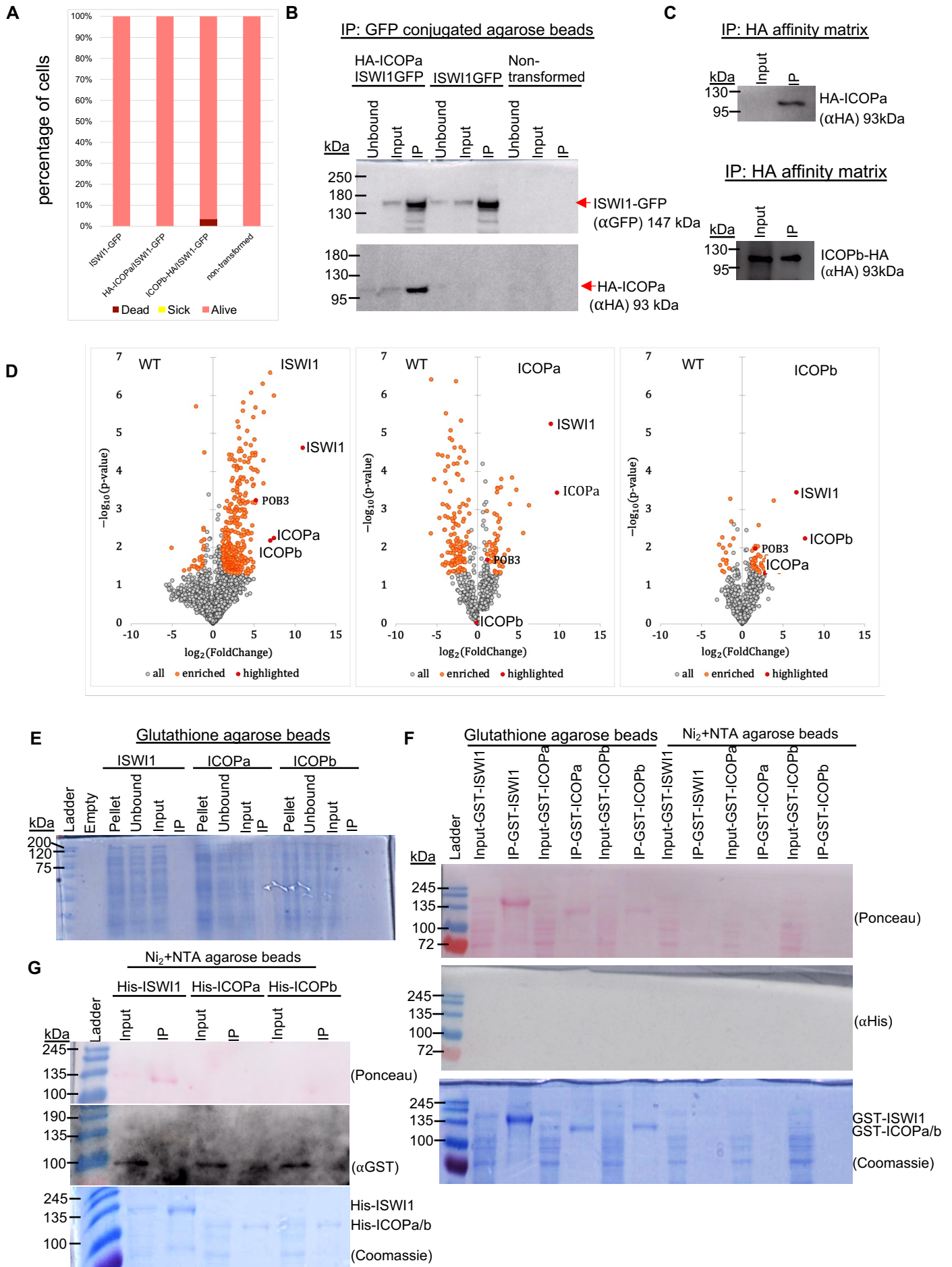
Supplemental Figure S2



Supplemental Figure S2: ICOP localization.

(A) & (B) Confocal fluorescence microscopy images of HA-ICOPa and ICOPb-HA localization at different developmental stages (A) or upon KDs (B). Maximum intensity projections of z-planes. Red: DAPI, Cyan: HA. Green arrow: MIC. White arrow: new MAC. Brightness and contrast in HA-channel are constant across all HA-ICOPa and across all ICOPb-HA images. Scale bar = 10 μ m.

Supplemental Figure S3

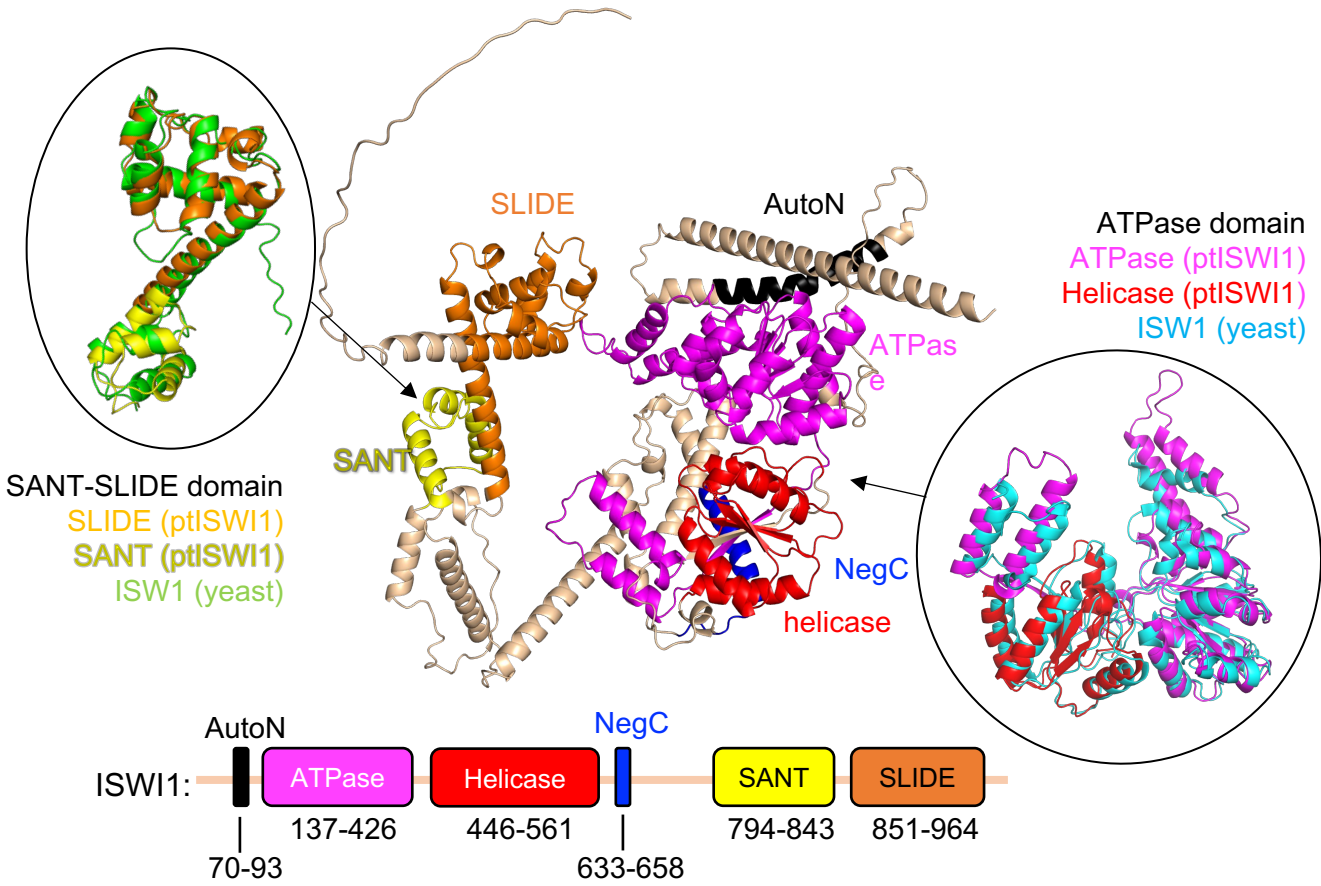


Supplemental Figure S3: ICOP paralogs interact with ISWI1.

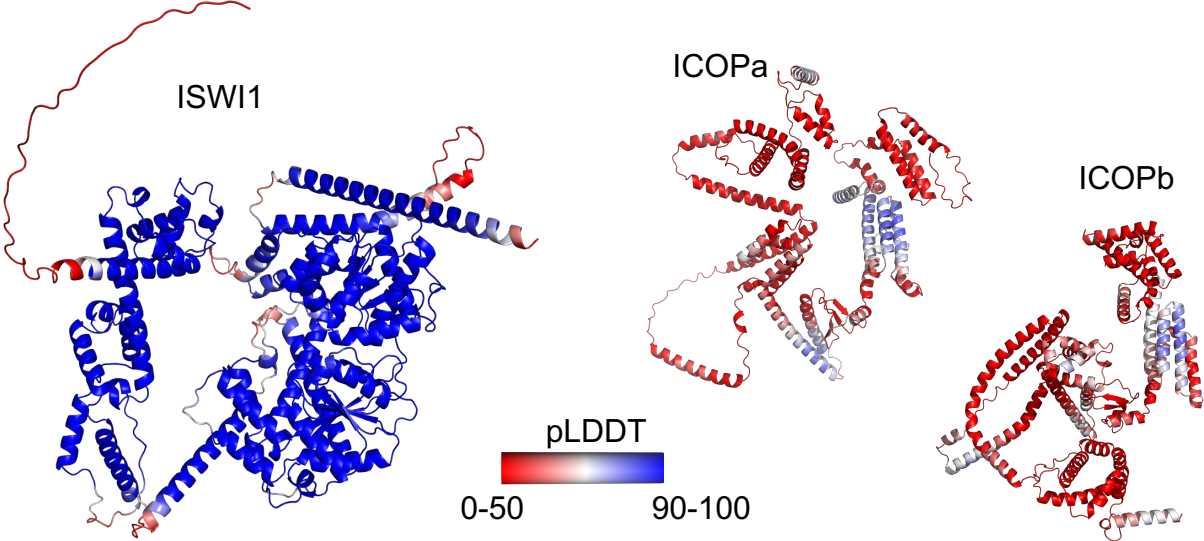
(A) Survival assay of F1 generation of the ISWI1-GFP, HA-ICOPa/ISWI1-GFP, and ICOPb-HA/ISWI1-GFP transformed cell lines, non-transformed cell lines are used as control. Alive (pink): normal division rate (4 divisions/day). Sick (red): slower division rate (<3 divisions/day). Dead (cayenne): no cells. (B) Western blot on co-IP of HA-ICOPa/ISWI1-GFP co-transformed, ISWI1-GFP transformed and non-transformed, wild-type *Paramecium*. (C) Western blot of co-IP of HA-ICOPa and ICOPb-HA overexpressed in paramecia. (D) Volcano plots showing protein enrichment of mass spectrometry (MS) analysis for ISWI1-GFP (left), HA-ICOPa (middle), and ICOPb-HA (right) co-IP. (E) to (F): Pulldowns on overexpressed recombinant proteins in *E. coli*. (E) Coomassie staining of untagged ISWI1, ICOPa and ICOPb. (F) Western blot and Coomassie staining of GST-tagged recombinant protein pulldowns; Ponceau-stained membranes probed with anti-His antibody. (G) Western blot and Coomassie staining of His-tagged recombinant proteins; Ponceau-stained membranes probed with anti-GST antibody.

Supplemental Figure S4

A



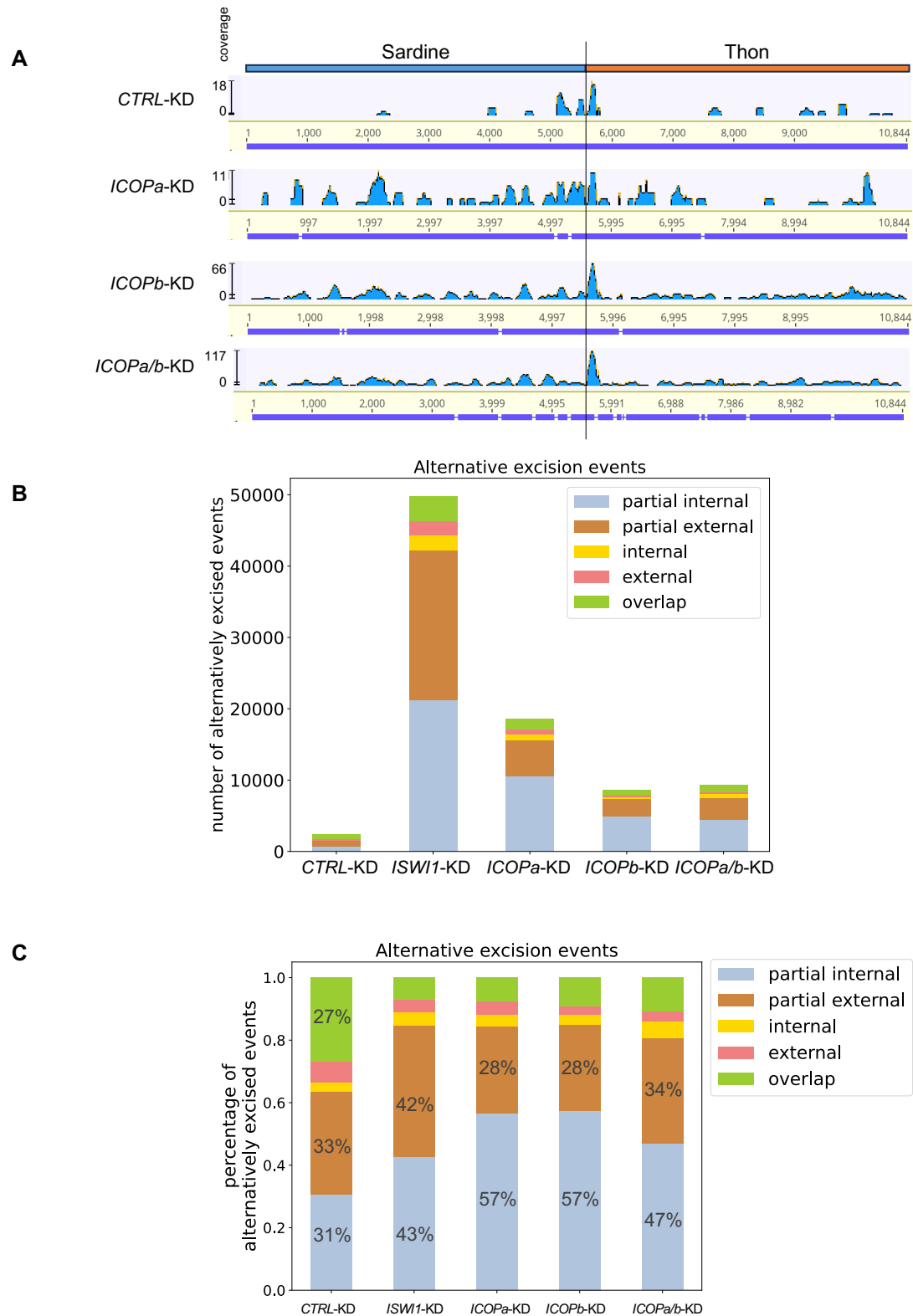
B



Supplemental Figure S4: ISWI1 and ICOP structure predictions.

(A) and (B) AlphaFold (version 2.2.0) structure predictions. (A) Domains in *Paramecium* ISWI1. ATPase and Helicase are superimposed with a published structure of N-terminal ISWI from yeast (PDB accession number 6JYL) (color: cyan) and SANT-SLIDE domains are superimposed with ISW1a (delATPase) from yeast (PDB accession number 2Y9Y) (color: green). (B) Structure prediction confidence for ISWI1, ICOPa, and ICOPb. Models are colored by predicted local distance difference test (pLDDT). pLDDT $\leq$ 50: red. pLDDT $\geq$ 90: blue.

Supplemental Figure S5

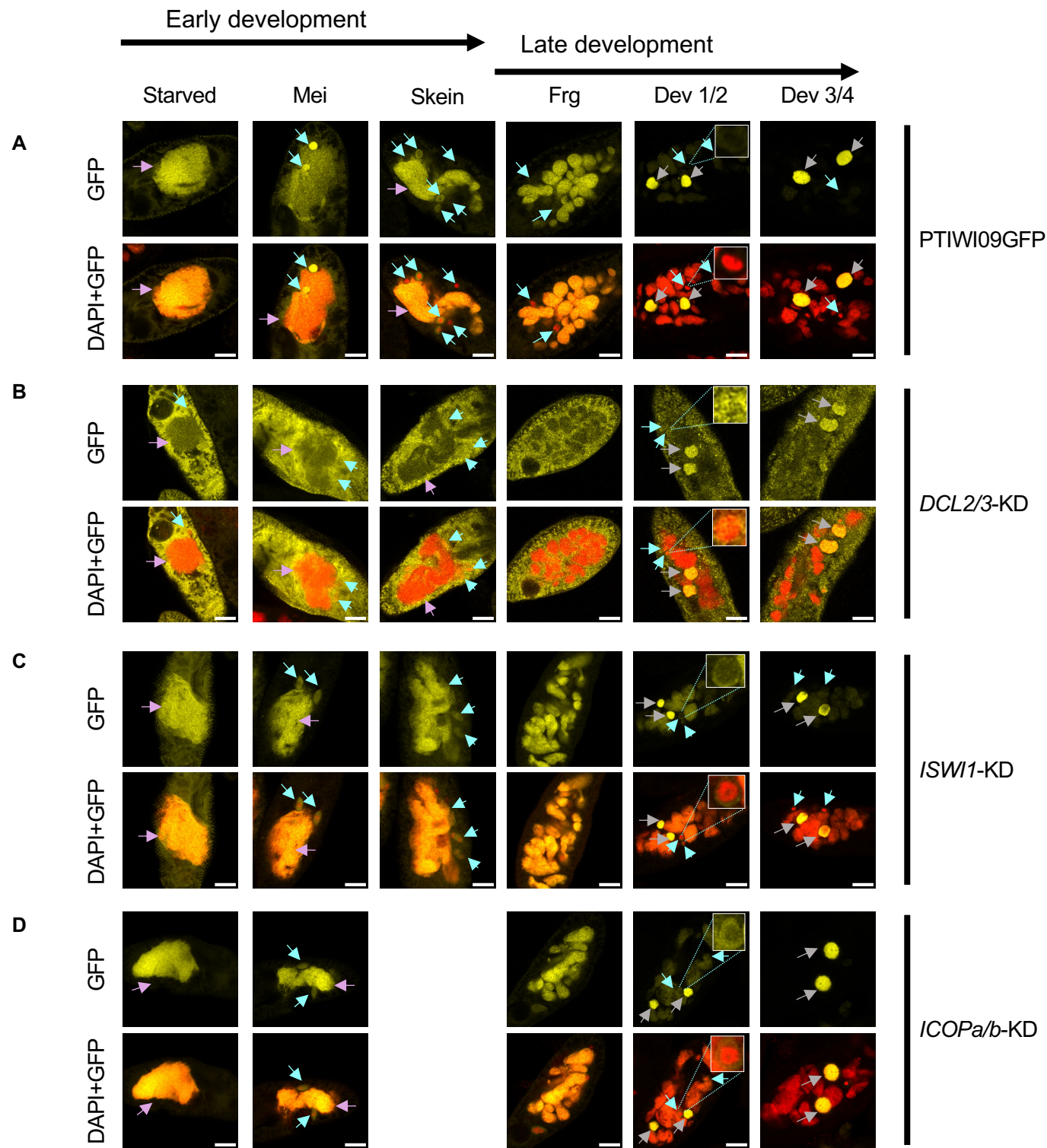


Supplemental Figure S5: DNA elimination events.

(A) Geneious screenshot of DNA-seq reads mapped to Sardine and Thon transposons (ENA HE774469) upon different knockdowns.

(B & C) Stacked bar graphs of alternative excision events in *ISWI1-KD*, *ICOPa-KD*, *ICOPb-KD* and *ICOPa/b-KD*. *ND7-KD* was used as a control (*CTRL-KD*). (B) Absolute and (C) relative abundance of alternative excision events occurring upon KDs.

Supplemental Figure S6



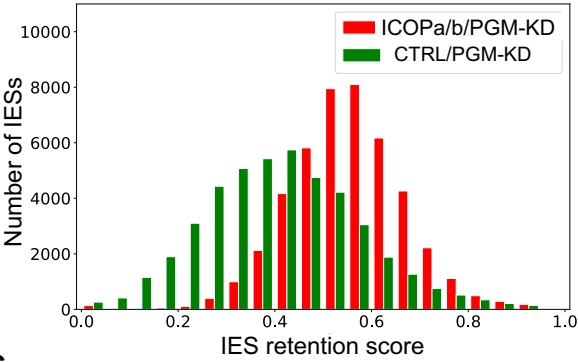
Supplemental Figure S6: Confocal microscopy images of PTIWI09GFP localization upon different knockdowns.

PTIWI09GFP localization without any knockdown (A), upon *DCL2/3*-KD (B), upon *ISWI1*-KD (C), upon *ICOPa/b*-KD (D). MIC is cropped, magnified, and contrast adjusted in Dev1/2 panel for better visualization. Red: DAPI. Yellow: GFP. Purple arrow: Maternal MAC. Cyan arrow: MICs. Grey Arrow: Developing MACs. All channels were optimized for the best visual representation. Scale bar = 10 μ m.

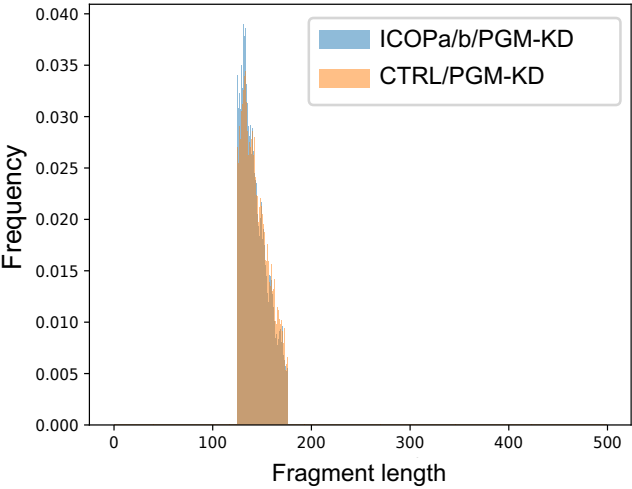
Starved: induction of autogamy (T0), Mei: MIC meiosis (T3-T5), Skein: beginning of macronuclear fragmentation (T4-T6), Frg: fragmentation of maternal MAC (T6-T10), Dev1/2: visible new MAC (T10-T12), Dev3/4: larger new MAC (T12-T16)

Supplemental Figure S7

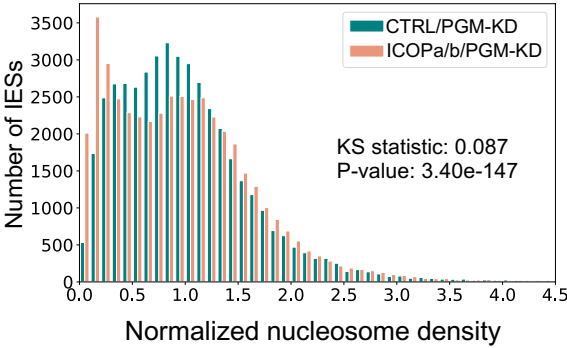
A



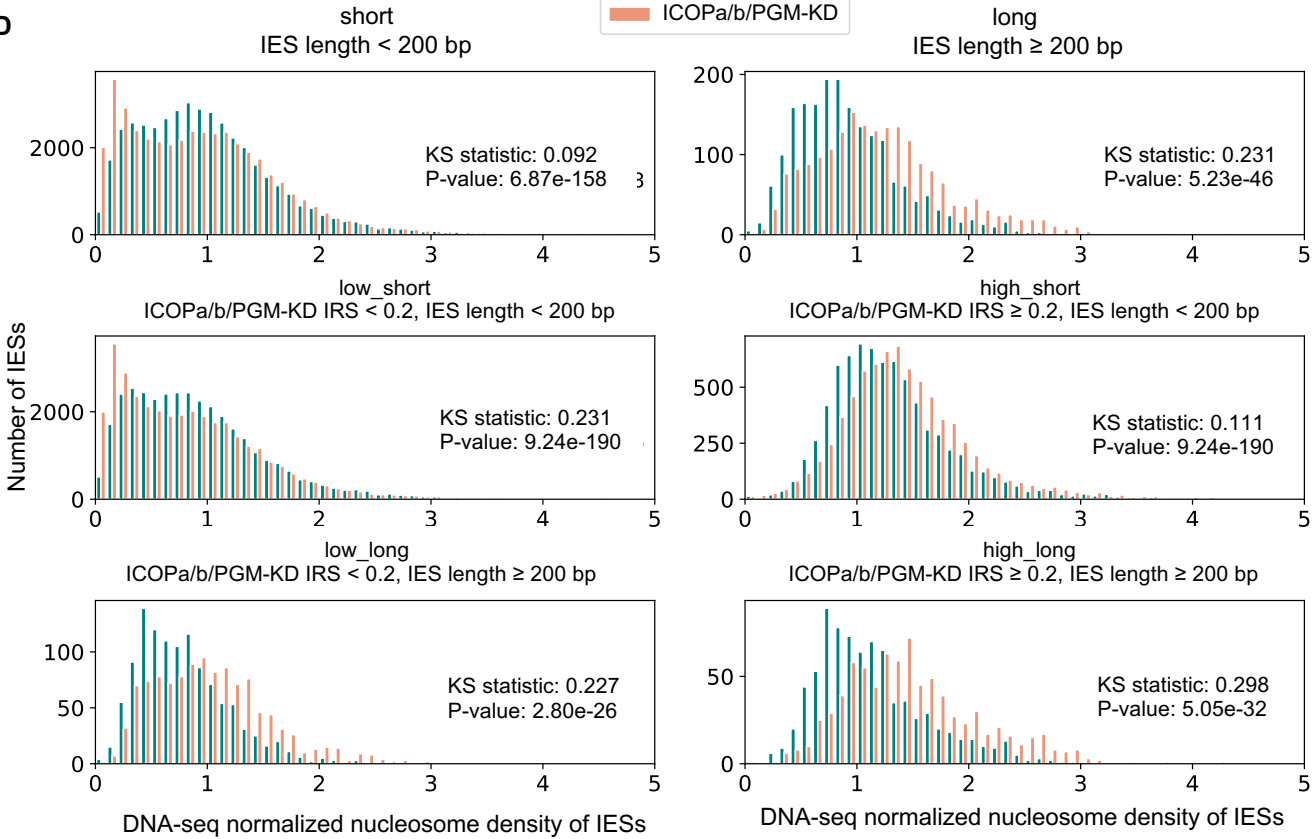
B



C



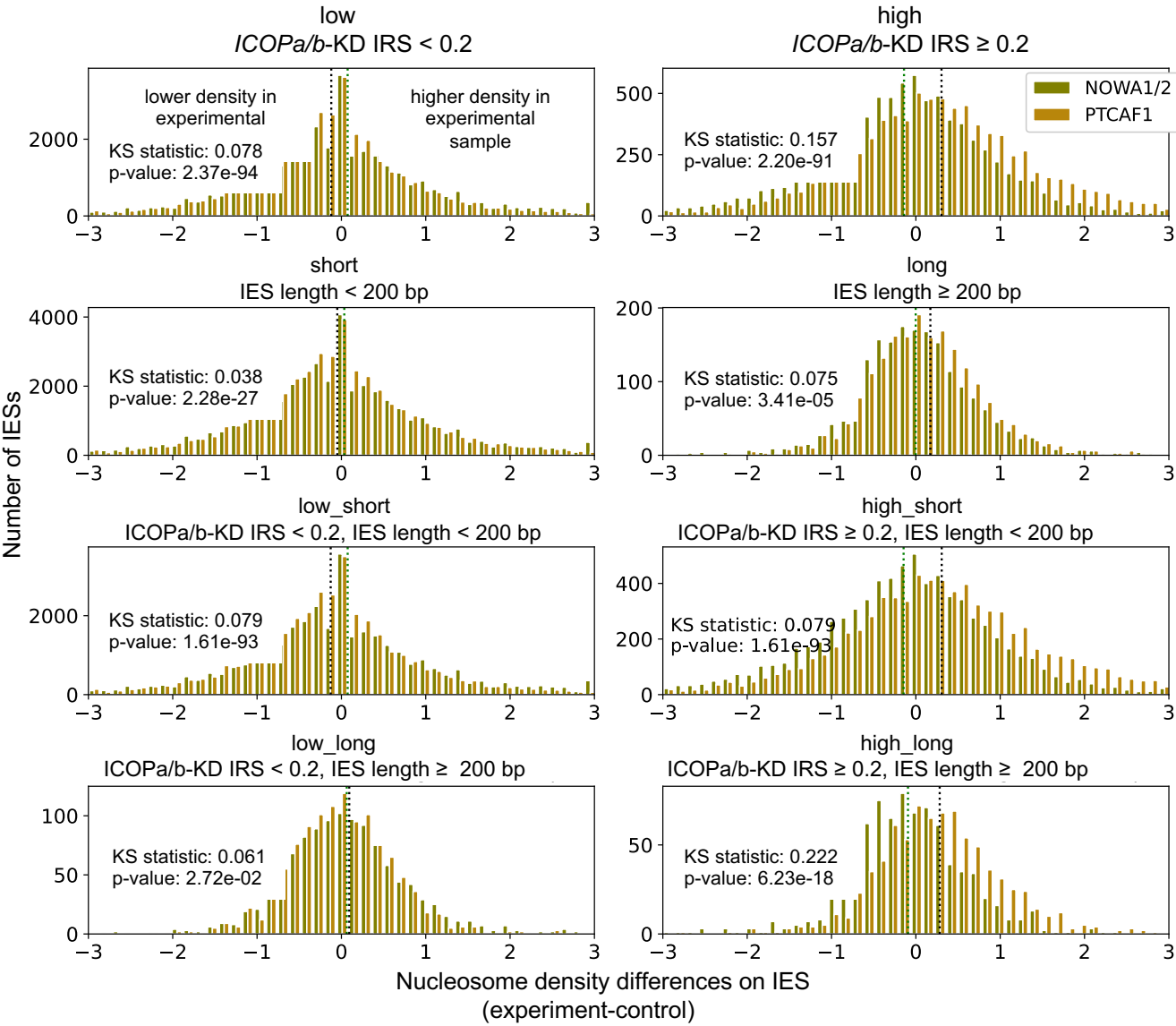
D



Supplemental Figure S7: Nucleosome densities for *ICOPa/b/PGM-KD* and *CTRL/PGM-KD*.

(A) IRS histogram for *ICOPa/b/PGM-KD* and *CTRL/PGM-KD*. (B) Size distribution of reads mapped to scaffold51_9 for *ICOPa/b/PGM-KD* and *CTRL/PGM-KD*. (C) Nucleosome densities of all IESs in *ICOPa/b/PGM-KD* and *CTRL/PGM-KD*. (D) Nucleosome densities of selected IES groups in *ICOPa/b/PGM-KD* and *CTRL/PGM-KD*. IESs were grouped by IES retention score (IRS) in *ICOPa/b-KD* (low: IRS < 0.2; high: IRS ≥ 0.2) and IES length (short: IES length < 200 bp; long: IES length ≥ 200 bp). IES group is given above the diagrams.

Supplemental Figure S8



Supplemental Figure S8: Nucleosome density differences for *NOWA1/2*/PGM-KD and *PTCAF1*/PGM-KD.

Comparison of *NOWA1/2*/PGM-KD and *PTCAF1*/PGM-KD nucleosome density differences in selected IES groups: IESs were grouped by IES retention score (IRS) in *ICOPa/b*-KD (low: IRS < 0.2; high: IRS ≥ 0.2) and IES length (short: IES length < 200 bp; long: IES length ≥ 200 bp). The specification for each IES group is given above the individual diagrams. Means are indicated as dashed lines (*NOWA1/2*/PGM-KD: green; *PTCAF1*/PGM-KD: black).