Materials and Methods

Fly genetics

To aid our analyses, we generated a rescue transgene, *P{Chd1$^+$}*, which encompasses full-length CHD1 cDNA under the control of its native promoter. A 3.2-kb fragment spanning 620 bp of the 5'-region, introns 1 and 2, exon 1 and part of exon 2 and a 0.5 kb fragment corresponding to the 3'-UTR of the *Chd1* gene were amplified from bacmid 23N6 and cloned in frame with the coding sequence of *Chd1* into pFastBacI-FCHD1 (*S1*). Hemeagglutinin (HA) and FLAG tags were inserted in frame with the C-terminus of CHD1. The CHD1 transgene was sequenced and cloned into pCaSpeR4. The construct was transformed into the germline of *y[1] w[67c23]* embryos. Two independent insertions on the second chromosome were recovered and mapped by iPCR to 47C3 and 49F5. *Df(2L)JS17 + P{Chd1$^+$}* chromosomes were produced by meiotic recombination in the female germline.

Custom *Df(2L)ED198(w$^+$)* deficiency that partially overlaps with *Df(2L)Exel7014* but does not uncover *Chd1* (Fig. 1A) was generated from corresponding RS element insertions with the help of the DrosDel Starter Kit. The original *l(2)23Cd[A7-4]* and *P{EPgy2}EY07345* stocks contain lethal mutations on the second chromosome that map outside of the *Chd1* locus. To outcross the unrelated lethal mutations, the corresponding alleles were maintained as heterozygotes with *Df(2L)ED198(w$^+$)* for several generations. Individual chromosomes from *w*$^-$ homozygotes were then balanced and tested for the absence of unrelated lethal mutations.

P-element excisions were performed by mating female homozygous *P{EPgy2}EY07345* flies with males carrying the source of transposase. Individual

mosaic males were then crossed to *Df(2L)ED198(w$^+$)* females, and second chromosomes of individual male progeny (*y*$^-$) from the latter cross (*ΔP{EPgy2}EY07345/Df(2L)ED198*) were assayed for reduced viability in combination with *Df(2L)JS17*. A total of 320 individual chromosomes were analyzed, and 8 putative *Chd1* imprecise excision alleles were obtained.

The putative *Chd1* alleles were balanced and assayed in genetic CHD1 rescue experiments. To this end, they were crossed inter se or with heterozygous balanced deficiency alleles with or without the rescue transgene *P{Chd1$^+$}*, and viabilities of the progeny were directly compared (Fig. S1). The *P{Chd1$^+$}* transgene rescues all phenotypes associated with the *Chd1* mutations.

The second chromosome insertion of *P{H3.3-FLAG}* (a gift of B. Loppin) was mapped by iPCR to 36A11. It was recombined with *Df(2L)Exel7014* in the female germline. For immunocytological analyses of H3.3 deposition in *Chd1* embryos, *Chd1[1]/Df(2L)Exel7014, P{H3.3-FLAG}* females were mated with males of various genotypes. Approximately 2,000 virgin females were used in each experiment.

To examine protamine removal from sperm DNA in *Chd1* mutants, *Chd1[1]/Df(2L)Exel7014* virgin females were mated with males carrying a Mst35Bb-GFP transgene (a gift of R. Renkawitz-Pohl).

To investigate the effects of CHD1 loss of function in the presence of maternal wild type CHD1, we generated a *UAS*-CHD1(K559R) transgene. The replacement of lysine 559 with arginine abolishes ATPase activity (*S2*) and, thus, should cause dominant negative effects. To generate the CHD1 wild type (*UAS*-CHD1(WT)) transgenic construct, a 6.5 kb Xho1-Not1 fragment of CHD1 cDNA was cloned into pUAST vector

with a modified polylinker. To generate ATPase-deficient *UAS*-CHD1(K559R) transgenic construct, the K559 codon (AAG) was replaced with an arginine codon (AGA) by PCR. The construct was transformed into the germline of *w[67c23]* embryos. To induce expression of the transgene in the early embryo, flies with homozygous insertions on the second or third chromosomes were crossed to homozygous flies that carry the *daughterless* driver on the third chromosome (*w; P{GAL4-da.G32}UH1*). To confirm the near complete elimination of its ATPase activity recombinant CHD1(K559R) polypeptide was synthesized in baculovirus and tested in ATPase and chromatin assembly assays in vitro.

When CHD1(K559R) was ectopically expressed under the control of a *da*-GAL4 driver, we observed lethality during embryonic or early larval stages. Noteworthy, the expression of the GAL4 driver is not induced in the embryo until later developmental stages, after the onset of zygotic transcription. Thus, CHD1 appears to have additional essential roles during development, subsequent to zygotic fusion in the embryo.

Indirect immunofluorescence and microscopy

For cytological analyses, embryos were collected 0–30 min or 0–4 hr after egg deposition (AED). Embryos were dechorionated and fixed in methanol as described (*S3*). DNA was stained either with 5 μg/ml PI or 1 μg/ml DAPI. Prior to PI staining, the embryos were incubated for 1 hr in a 2 mg/ml RNAse A solution at 37°C (*S4*). For IF experiments, embryos were collected in 30-min intervals and stored at 4°C for up to 2 hr before fixation with methanol. The fixation and immunostaining were done exactly as described (*S4*). Vectashield-mounted preparations were observed under a Zeiss

Axioscope 2. For each experiment, more than 500 embryos were observed. Images were processed using IP Lab and Photoshop. Mouse monoclonal anti-FLAG antibody M2 (Sigma) was used at 1:1,000. Rabbit polyclonal anti-GFP antibody (Clontech) was used at 1:40. ChIP grade rabbit polyclonal antibody to the C-terminus of human histone H3 (Abcam) was used at 1:500. Appropriate secondary antibodies conjugated with Alexa-Fluor 350 or 488 (Molecular Probes) were used at 1:1,000.

In pronuclear apposition stage embryos, male and female pronuclei are typically positioned very close to each other. When *Chd1* null embryos in pronuclear apposition stage are stained with the H3 antibody, the glare from the strong H3 signal in the female pronucleus does not allow to discern finer details of the staining pattern in the adjacent more weakly-stained male pronucleus (Fig. 3B). Rarely, the male and female pronuclei are positioned sufficiently far from each other or in distinct focal planes. In that case, H3 staining of the male pronucleus clearly resembles that of H3.3-FLAG (compare Figs. 3D and S5).

PCR analysis

The deficiencies *Df(2L)Chd1[1]* and *Df(2L)Chd1[2]* were mapped by iPCR with primers from within the coding sequence of *Chd1* and sequenced. The complete *Chd1* transcription unit from *Chd1[3]/Df(2L)JS17* flies was also amplified and sequenced.

For determination of haploid chromatin content in *Chd1* mutant embryos, *Chd1[1]/Df(2L)Exel7014* or *Chd1[2]/Df(2L)Exel7014* females were mated to *+/CyO Kr::GFP* males. Embryos were collected at 0–4 hr AED, dechorionated, and genomic DNA was prepared from pools of ~200 embryos by phenol extraction. Various DNA

dilutions were used for multiplex PCR amplification of a 529 bp fragment of the Asf1 gene and a 455 bp fragment of the GFP gene.

All primer sequences and PCR conditions are available upon request.

Western blot analysis of *Chd1* mutant alleles

Embryos from *Chd1[1]/CyO, Chd1[2]/CyO* and *Chd1[3]/CyO* flies were collected at 0-4 h AED, dechorionated and processed as follows: Embryos (approximately 0.5 g) were suspended in 300 μl 2X SDS-PAGE sample buffer, homogenized with a small pestle in a 1.5 ml Eppendorf tube and boiled for 5 min. The samples were then centrifuged, and aliquots of supernatants were subjected to SDS-PAGE and subsequent western blotting. Blots were incubated with an antibody against the N-terminus (residues 79–276) of CHD1 at a dilution of 1:50,000.

Protein expression analysis during embryonic development

Embryos from wild type flies were collected at 0–1 hr AED and aged at 25°C for the stated periods of time. Dechorionated embryos were processed for western analysis as described above. Blots were incubated with anti-CHD1 and anti-ISWI antibodies as described below. Equal loading was confirmed by electrophoresis and subsequent Coomassie Brilliant Blue staining of 1/5 of the sample volumes used for western analysis.

Anti-FLAG immunoprecipitation

Cytoplasmic extracts of wild type and FLAG-HIRA expressing embryos (0–12 hr AED) were prepared as follows. Embryos where dechorionated and homogenized in

Buffer A (15 mM Tris-HCl pH 7.5, 15 mM NaCl, 60 mM KCl, 340 mM sucrose, 15 mM spermidine, 15 mM spermine, 2 mM EDTA, 0.5 mM EGTA, 0.25 mM PMSF, 0.1% β-mercaptoethanol), at 4 ml per g embryos. The homogenate was filtered through Miracloth and cleared by centrifugation at 8000 *g*. The supernatant was supplemented with NaCl to 140 mM and centrifuged again. The extract was either used immediately or stored at –80°C.

For co-immunoprecipitation experiments, 500 μl of cytoplasmic extracts from FLAG-HIRA or control embryos were incubated in batch with 50 μl anti-FLAG-M2 agarose (Sigma) overnight at 4°C with gentle agitation. Subsequently, beads were washed twice with 1 ml Buffer A + 150 mM NaCl and 3 times with 1 ml Buffer A + 250 mM NaCl. Finally, the beads were suspended in 2X SDS-PAGE sample buffer. Aliquots of input, supernatant after antibody incubation and beads were subjected to SDS-PAGE and western blotting. Blots were incubated sequentially with anti-FLAG antibody (Sigma) diluted 1:1,000, anti-CHD1 antibody (*S5*) diluted 1:2,000 and anti-ISWI antibody (*S6*) diluted 1:3,000. Secondary antibodies were HRP-conjugated anti-mouse IgG and anti-rabbit IgG (GE Healthcare) diluted 1:10,000.

Supplementary References

S1. A. Lusser, D. L. Urwin, J. T. Kadonaga, *Nat. Struct. Mol. Biol.* **12**, 160 (2005).

S2. R. Deuring *et al.*, *Mol. Cell* **5**, 355 (2000).

S3. W. F. Rothwell, W. Sullivan, *Fluorescent analysis of Drosophila embryos*. W. Sullivan, M. Ashburner, R. S. Harwley, Eds., *Drosophila* Protocols (Cold Spring Harbor Laboratory Press, New York, 2000), pp. 141-159.

S4. B. Loppin, F. Berger, P. Couble, *Chromosoma* **110**, 430 (2001).

S5. D. G. Stokes, K. D. Tartof, R. P. Perry, *Proc. Natl. Acad. Sci. USA* **93**, 7137 (1996).

S6. T. Ito, M. Bulger, M. J. Pazin, R. Kobayashi, J. T. Kadonaga, *Cell* **90**, 145 (1997).

Table S1. Developmental progression in *Chd1* mutant embryos.

[a] Embryos, 0 – 4 hr after egg deposition (AED), were collected and stained with DAPI.

[b] The number of nuclei as well as phenotypic appearance were used as measures of progression of development.

[c] The sample size was 400 stained embryos each from wild-type and *Chd1[3]/Df(2L)Exel7014* mothers.

Supplementary Figure Legends

Fig. S1. Viability of *Chd1* mutant alleles. Flies bearing homozygous *Chd1[1], Chd1[2]* or *Chd1[3]* alleles exhibit a decrease in viability of 67 – 98% (white bars). Heterozygous combination of individual mutant alleles with *Df(2L)Exel7014* (green bars) or *Df(2L)JS17* (yellow bars), which uncover *Chd1*, results in viability decrease of 21 – 87% of the expected homozygous progeny. Viability of all three mutant alleles is restored in heterozygous combinations with a chromosome that contains *Df(2L)JS17* and a *P{Chd1$^+$}* transgene insertion encoding wild-type CHD1-HA-FLAG (orange bars).

Fig. S2. The absence of CHD1 in the egg does not affect protamin B removal from sperm chromatin. Males that carry GFP-tagged Mst35Bb (protamin B) were mated to *Chd1* null females. Protamin B (blue) is present in paternal chromatin of *Chd1[1]/Df(2L)Exel7014* eggs shortly after sperm entry into the egg (left) but is undetectable during subsequent migration towards the center of the egg (middle) or during pronuclear apposition (right). H3.3-FLAG IF signal (green) is used to discern the male pronucleus. Scale bars, 20 μm.

Fig. S3. Molecular model of ATP-dependent nucleosome assembly by CHD1 and HIRA. H3.3- and H4-containing histone tetramers are delivered to the male pronucleus by the HIRA chaperone complex. The histones are then deposited on the DNA by the ATP-utilizing molecular motor protein CHD1.

Fig. S4. CHD1 and ISWI motor proteins are expressed during early embryonic development but differ in their ability to physically interact with the H3.3-specific histone chaperone HIRA. (**A**) Whole cell extracts were prepared from wild type embyos of indicated ages, subjected to SDS-PAGE and western blotting and hybridised with antibodies against CHD1 (top panel) and ISWI (middle panel). Consistent protein loading was confirmed by SDS-PAGE and Coomassie Brilliant Blue staining of cognate samples (bottom panel). (**B**) Cytoplasmic extracts were prepared from embryos expressing a FLAG-HIRA transgene (left panels) or wild type embryos (right panels), and immunoprecipitation was performed with anti-FLAG agarose. Aliquots of input (input; 1%), supernatant after antibody incubation (sup; 1%) and protein released from the agarose beads by boiling in the sample buffer (beads; 25%) were tested by western blot for co-immunoprecipitation of CHD1 and ISWI with FLAG-HIRA.

Fig. S5. Histone H3 incorporation into the male pronucleus is impaired in *Chd1* null embryos. Staining with the H3 antibody produces a speckled pattern in the periphery of the male pronucleus that becomes visible when the female pronucleus is positioned in a different focal plane (left panels) or far from the male pronucleus (right panels). Arrows, male pronuclei (m). Arrowheads, female pronuclei (f). Labeling above the panels refers to genotype of mothers. Red, PI; green, H3. Scale bars, 10 μm.

Table S1. Developmental progression in *Chd1* mutant embryos.

Developmental Stage[a,b]	**Typical Developmental Timing in the *wild type***	**%, *wt*[c]**	**%, *Chd1*[c]**
Absent/degraded nuclei	Unknown	8%	31%
1 – 4 nuclei/pronuclei/polar body	0 – 30 min	24%	56%
Division cycles 3 – 13	0.5 – 2.5 hr	29%	9%
Cellular blastoderm or later	> 2.5 hr	39%	4%

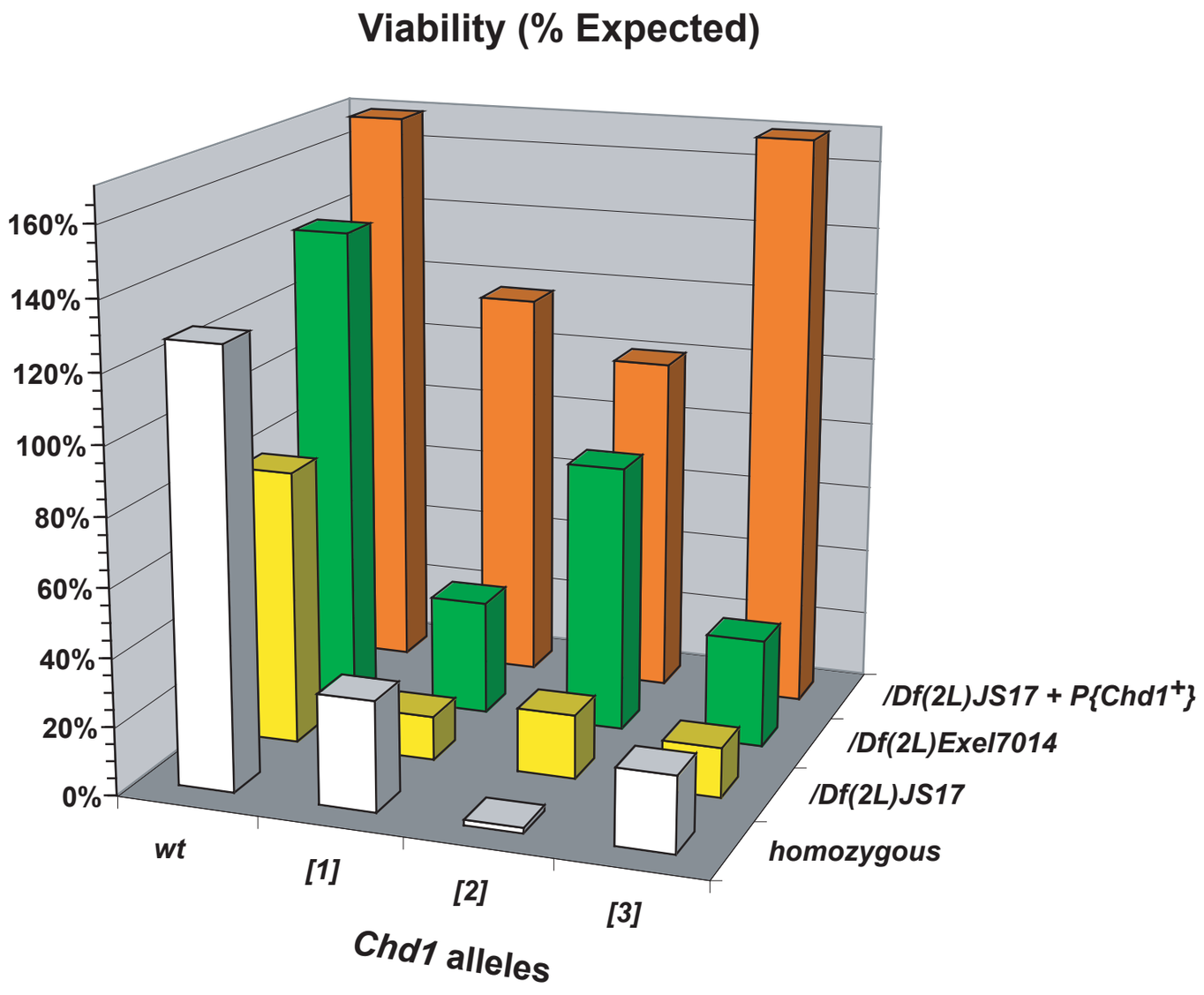

Figure S1
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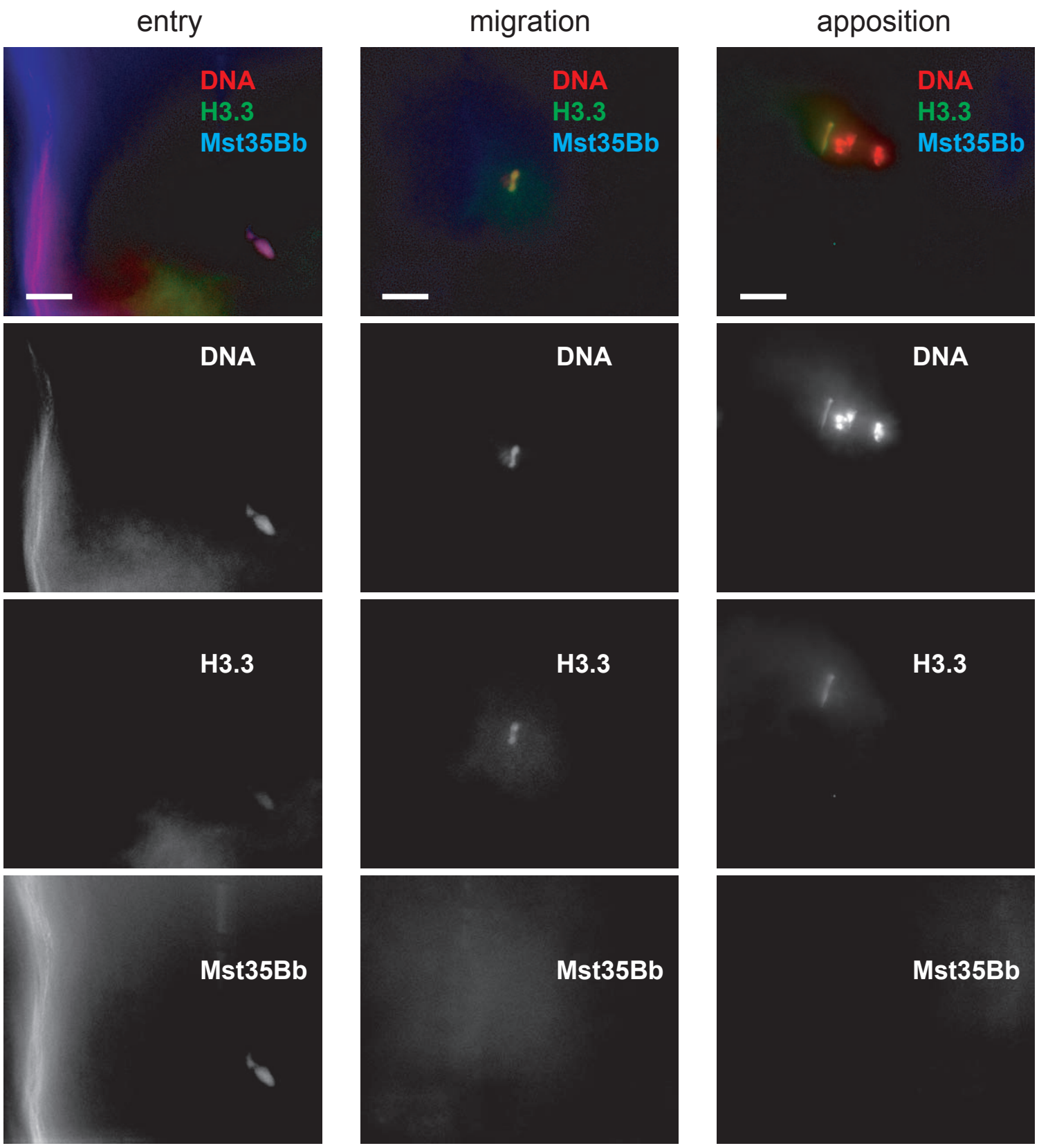

Figure S2
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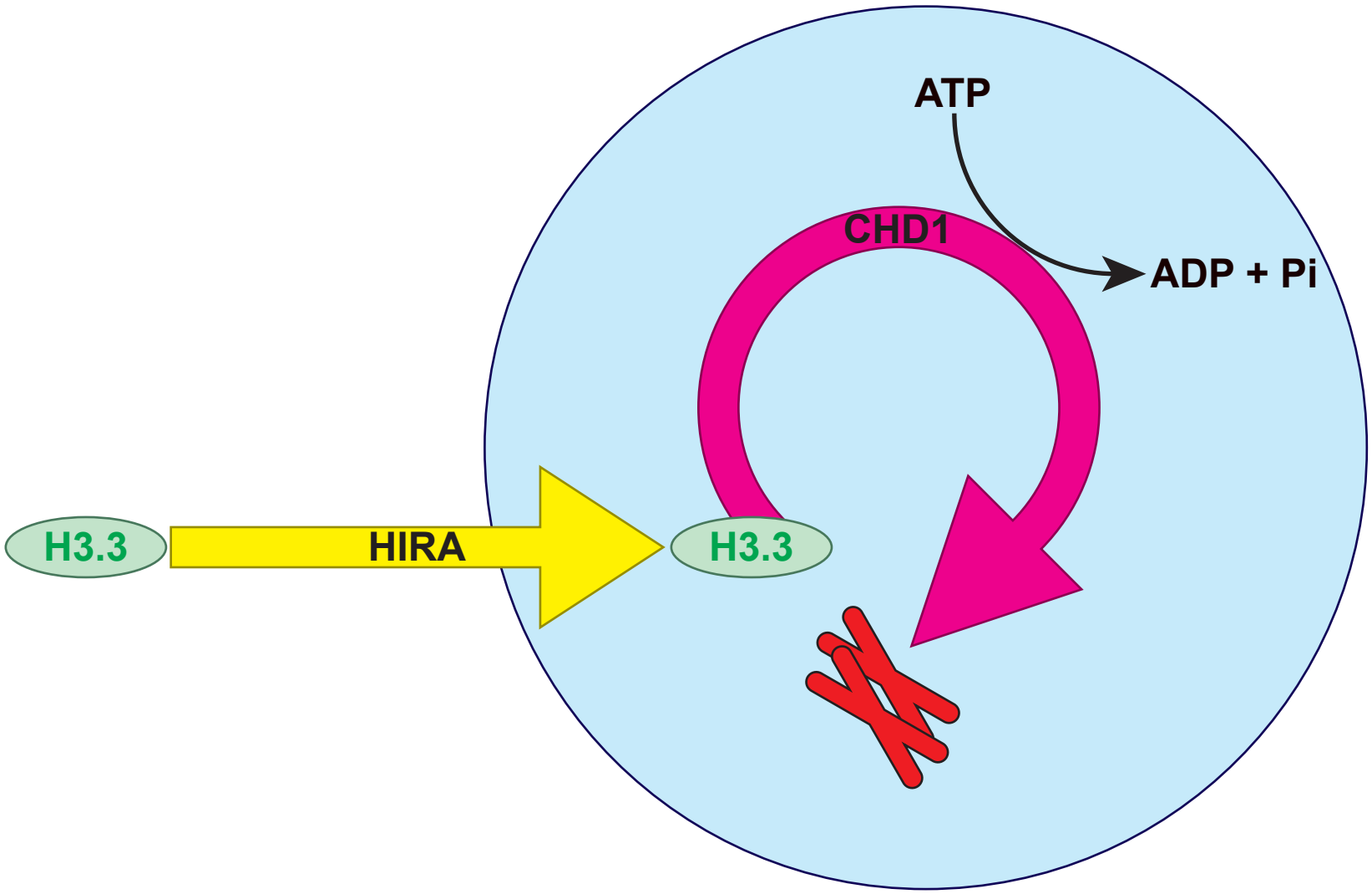

Figure S3
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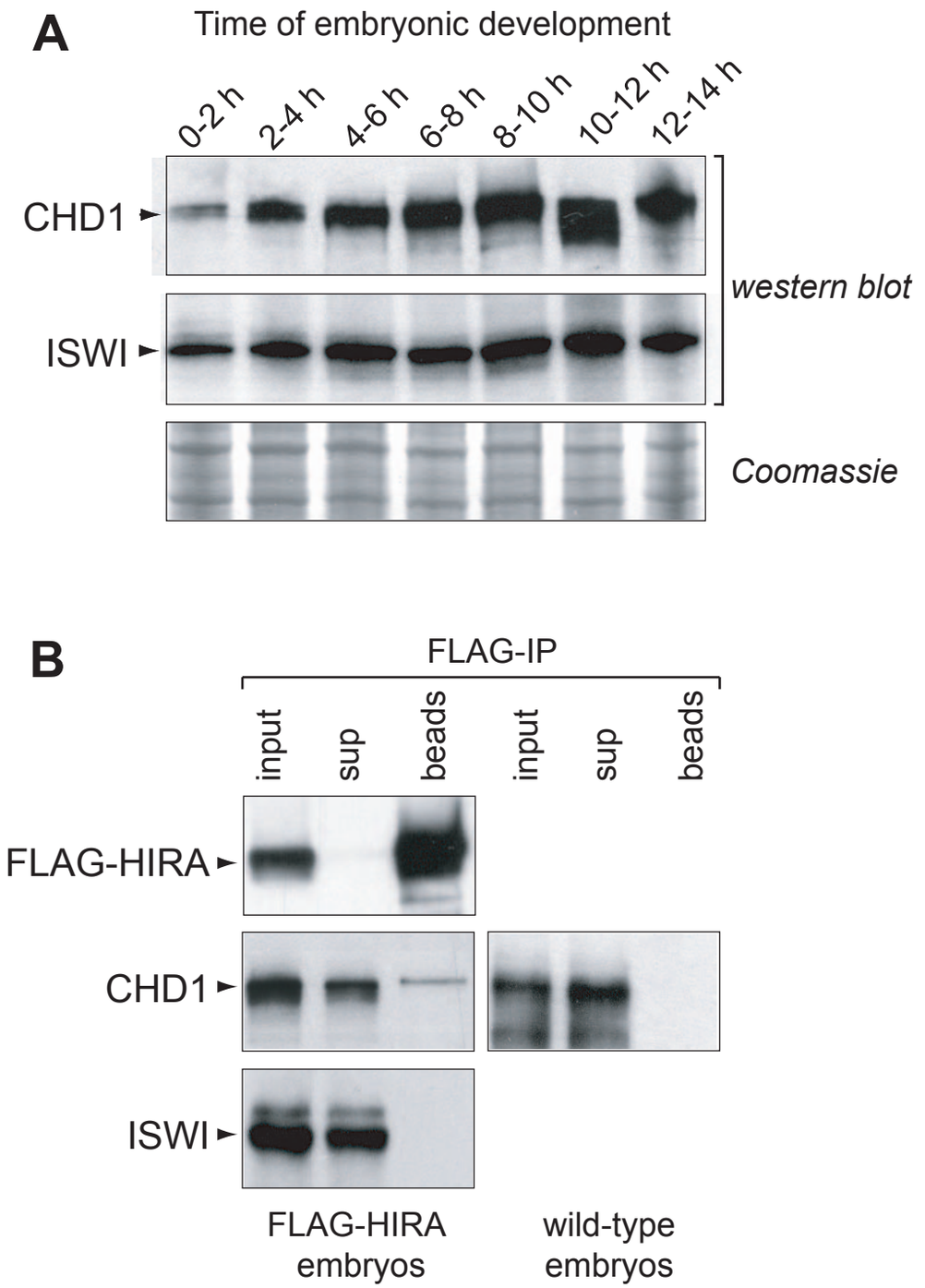

Figure S4
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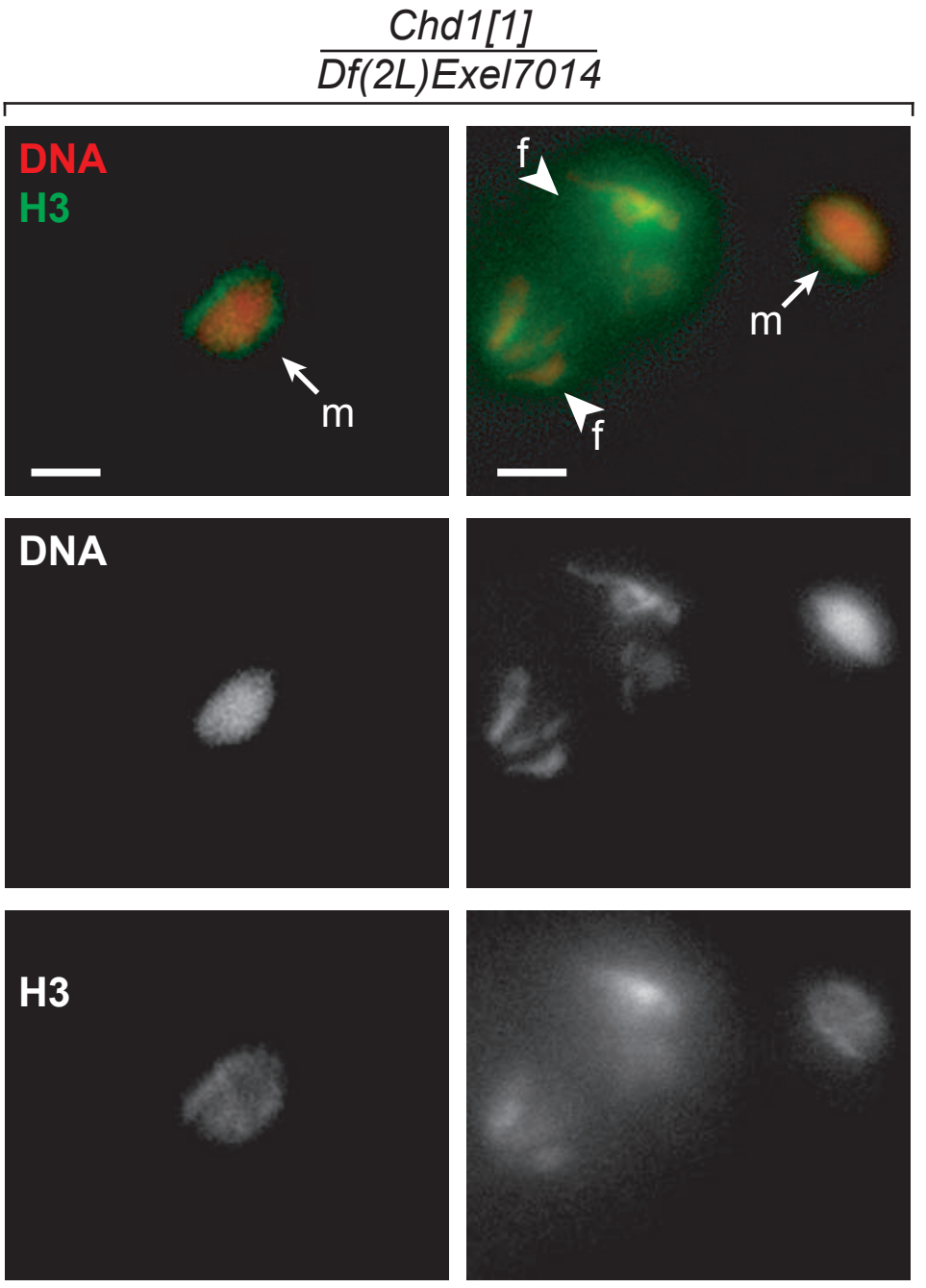

Figure S5
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