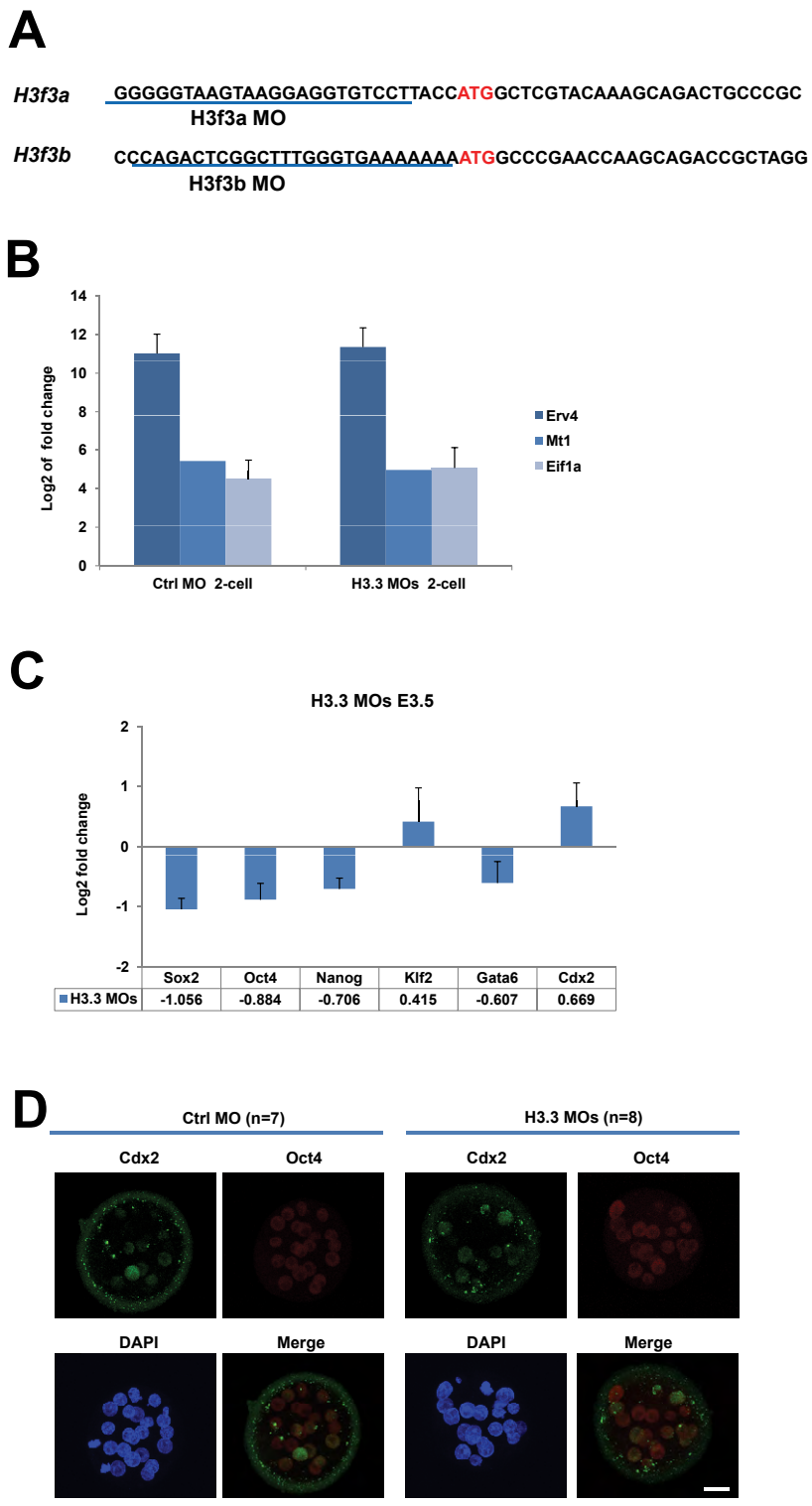

Fig. S1. Design of H3.3 morpholinos and analysis of effects of H3.3 knockdown on gene expression. (**A**) Sequences for morpholino oligos designed to target 5′ UTR sequences of the *H3f3a and H3f3b* genes. Red ATG characters refer to the start codon. (**B**) The expression of diagnostic markers of ZGA is not altered in H3.3-deficient embryos. The expression level of *Erv4*, *Mt1* and *Eif1a* was analyzed in Ctrl MO- and H3.3 MO-treated two-cell embryos by qRT-PCR. Values were standardized to *Hprt* and compared with zygotes. All three genes are highly induced at the two-cell stage relative to the zygote in control and H3.3-deficient embryos, indicative of normal ZGA. (**C**) The expression of genes involved in lineage commitment at the morula stage is not significantly altered in H3.3-deficient embryos. The expression level of markers of ICM/epiblast (*Oct4*, *Sox2*, *Nanog*, *Klf2*), primitive endoderm (*Gata6*) and throphectoderm (*Cdx2*) was analyzed in H3.3 MO-treated embryos at the morula stage by qRT-PCR. Values were normalized to *Hprt* and compared with Ctrl MO embryos. Gene expression differences are minor at best (less than twofold) and not always consistent between different embryo batches (data not shown). (**D**) Oct4 and Cdx2 are normally expressed in H3.3 MO embryos. Immunofluorescence of Cdx2 (green) and Oct4 (red) in Ctrl MO and H3.3MOs morulae. Scale bar: 20 μm. Error bars indicate s.d.

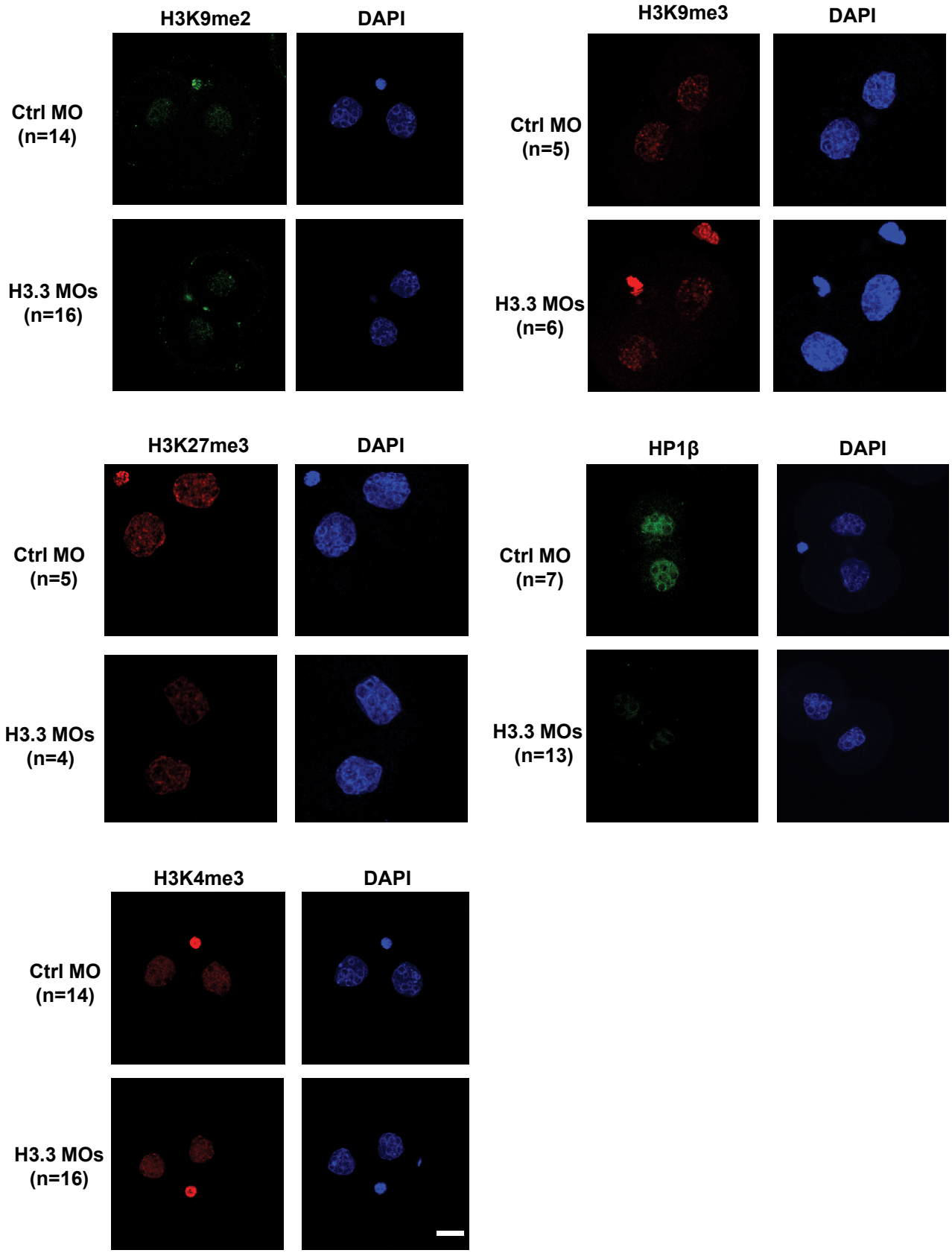

Fig. S2. Analysis of additional histone marks in H3.3-deficient embryos. Immunofluorescence images of H3K9me2, H3K9me3, H3K27me3, HP1β and H3K4me3 in Ctrl MO- and H3.3 MO-treated embryos at the two-cell stage. Scale bar: 20 μm.

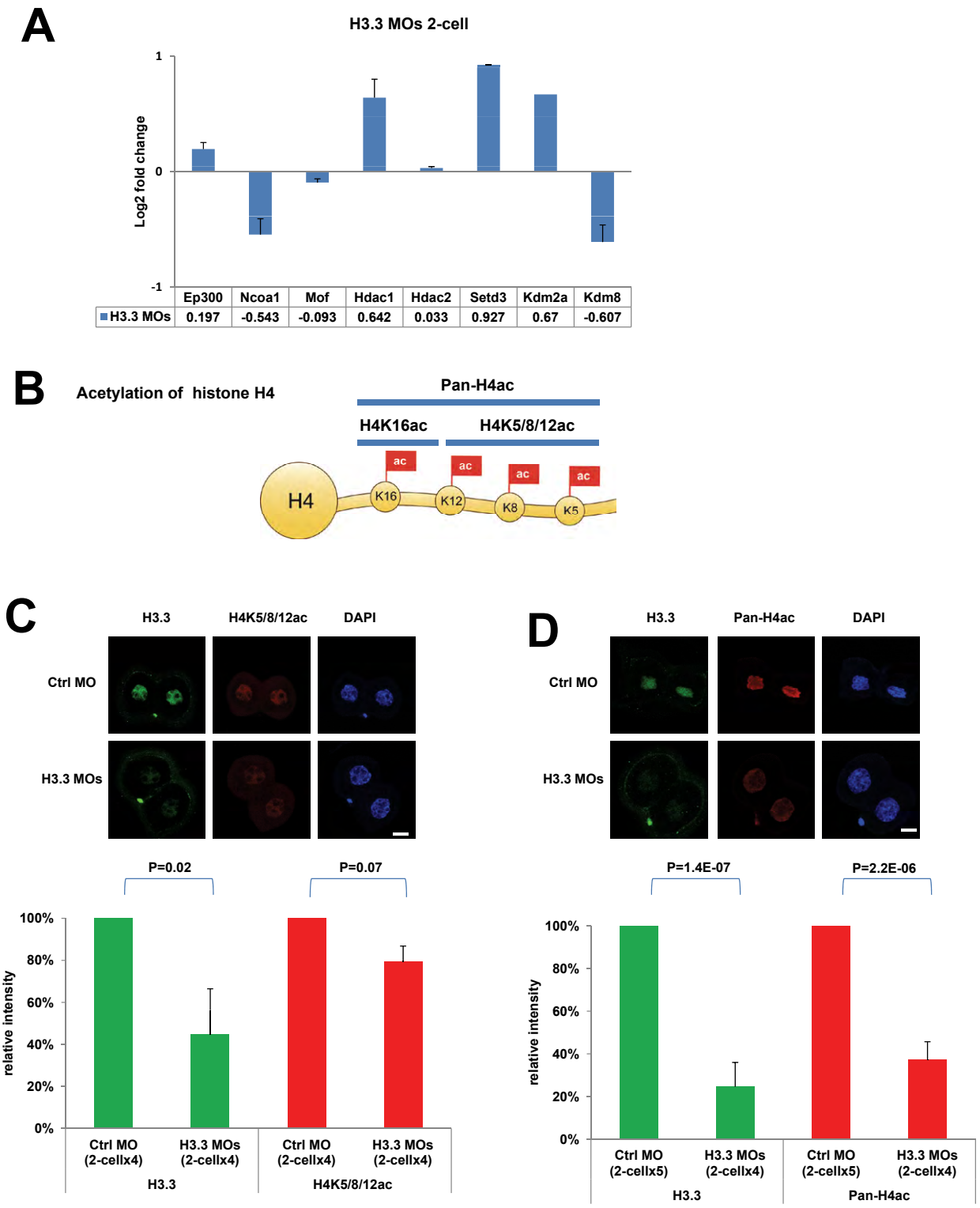

Fig. S3. H4 acetylation loss in H3.3-deficient embryos is restricted to H4K16. (**A**) The expression of enzymes involved in imposing or removing H4K16ac and H3K36me2 is not significantly altered in H3.3-deficient embryos. The expression level of H4 acetyltransferases (*Ep300* and *Ncoa1*), an H4K16-specific acetyltransferase (*Mof*), H4 deacetylases (*Hdac1* and *Hdac2*), H3K36-specific methyltransferase (*Setd3*) and H3K36- specific demethylases (*Kdm2a* and *Kdm8*) was analyzed in H3.3 MO-treated two-cell embryos by qRT-PCR. Values were standardized to *Hprt* and compared with Ctrl MO embryos. (**B**) Schematic of the H4 tail with lysines capable of undergoing acetylation and the antibodies that recognize them indicated. The pan-H4ac antibody can recognize all four acetyl-lysine sites; the H4K5/8/12ac antibody can recognize all sites except K16; and the H4K16ac antibody is specific to K16. (**C**) No significant changes in H4 acetylation are detected in H3.3-deficient embryos outside of the K16 residue. Immunofluorescence (upper panel) and quantification (lower panel) of H3.3 (green) and H4K5/8/12ac (red) in Ctrl MO and H3.3 MO embryos at the two-cell stage. Scale bar: 20 μm. (**D**) A pan-H4 acetylation antibody shows a significant decrease in staining in H3.3-deficient embryos. Immunofluorescence (upper panel) and quantification (lower panel) of H3.3 (green) and pan-H4ac (red) in Ctrl MO and H3.3 MO embryos at the two-cell stage. Scale bar: 20 μm. Error bars indicate s.d.

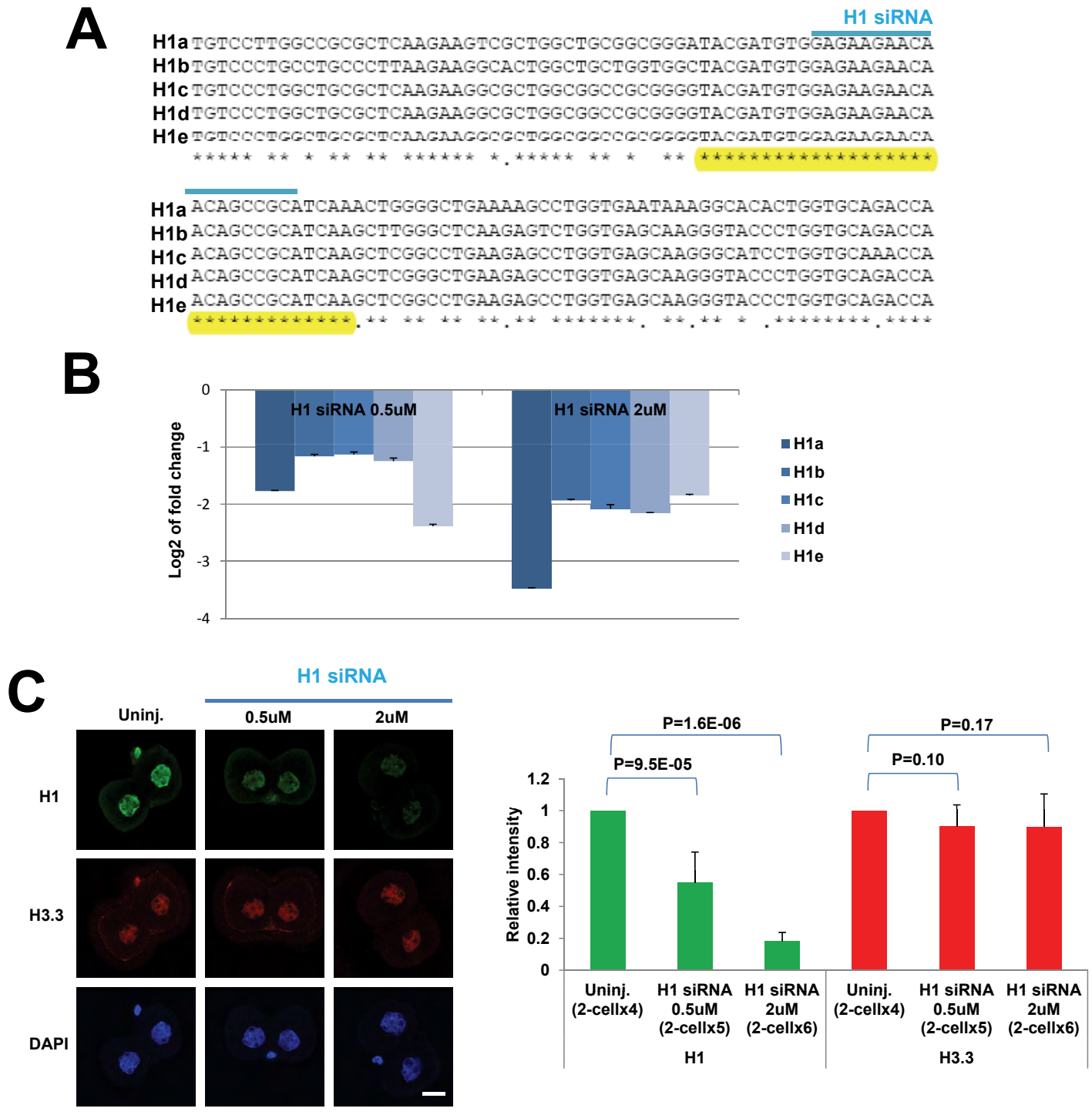

Fig. S4. Design and validation of the H1 siRNA used for rescue of H3.3-deficient embryos. (**A**) Alignment of H1 isotypes and design of pan-H1 siRNA. Multiple sequence alignment was carried out using Clustal Omega (European Bioformatics Institute). The region highlighted in yellow indicates a sequence identical among all five H1 isotypes, and the blue line indicates the H1 siRNA designed and used in this manuscript. (**B**) Validation of H1 mRNA knockdown upon siRNA treatment. The expression level of the five H1 isotypes was analyzed in H1 siRNA (0.5 and 2 μM)-injected two-cell embryos by qRT-PCR. Values were standardized to *H2A.1* and compared with NT siRNA (0.5 μM)-injected two-cell embryos. (**C**) Validation of H1 protein knockdown upon siRNA treatment. Immunofluorescence (left panel) and quantification (right panel) of H1 (green) and H3.3 (red) in Uninj. and H1 siRNA (0.5 and 2 μM) embryos at the two-cell stage. Scale bar: 20 μm. Error bars indicate s.d.

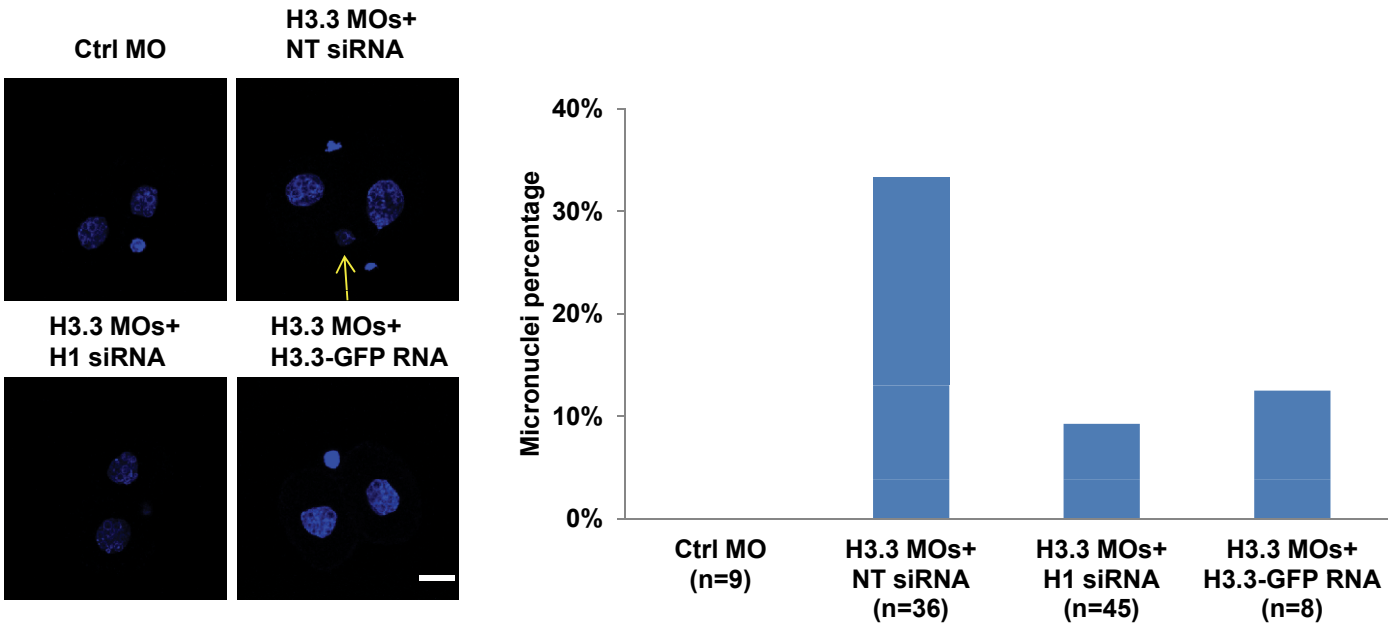

Fig. S5. The micronuclei phenotype in H3.3-deficient embryos can be rescued by H1 knockdown. Left panel: Representative DNA (DAPI) images of two-cell embryos injected at the zygote stage with Ctrl MO, H3.3 MOs plus NT siRNA, H3.3 MOs plus H1 siRNA, or H3.3 MOs plus H3.3-GFP mRNA. Arrow indicates micronucleus. Scale bar: 20 μm. Right panel: Quantification of incidence of micronuclei in two-cell embryos injected at the zygote stage with Ctrl MO, H3.3 MOs plus NT siRNA, H3.3 MOs plus H1 siRNA, or H3.3 MOs plus H3.3-GFP mRNA.

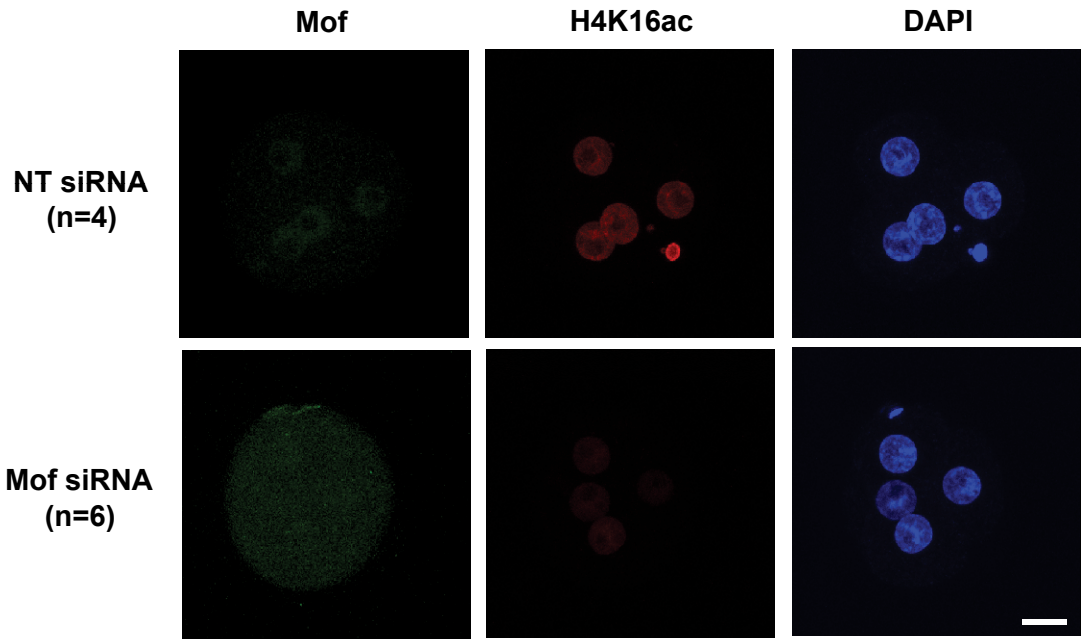

Fig. S6. Validation of Mof and H4K16ac knockdown upon Mof siRNA treatment. Immunofluorescence of Mof (green) and H4K16ac (red) in NT siRNA- and Mof siRNA-injected embryos at the four-cell stage. Scale bar: 20 μm.

Table S1. Primers used for quantitative RT-PCR

Gene	Forward (5′-3′)	Reverse (5′-3′)
H2A.1	ACATGGCGGCGGTGCTGGAGTA	CGGGATGATGCGCGTCTTCTTGTT
Hprt	GCTTGCTGGTGAAAAGGACCTCTCGAAG	CCCTGAAGTACTCATTATAGTCAAGGGCAT
Erv	ACATGAACAAAGTGGCCATGGTGG	AGTGTTGGTTTCTGCTGTTGGCAG
Mt1	TCCTGCAAGAAGAGTGAGTTGGGA	AGACAATACAATGGCCTCCGGGAA
Eif1a	CAACACTGTTTGCTGCCTGTGGAT	ACAGCAGCTGAGACTCCTTTCCAA
Sox2	AGCCTCCAGCAGATGCAAGA	GCACTTCATCCTTTGGTTTTGAA
Oct4	AGCCGACAACAATGAGAACC	TGGTCTCCAGACTCCACCTC
Nanog	GCTCAGCACCAGTGGAGTATCC	TCCAGATGCGTTCACCAGATAG
Klf2	CACCAAGAGCTCGCACCTAA	TTTCGGTAGTGGCGGGTAAG
Gata6	TTAACACTGATTGCTGCAACG	GTTCATCGTAACGTGGCTGA
Cdx2	CCTGCGACAAGGGCTTGTTTAG	TCCCGACTTCCCTTCACCATAC
Ep300	GTTGCTATGGGAAACAGTTATGC	TGTAGTTTGAGGTTGGGAAGG
Ncoa1	AGGAGTGATAGAGAAGGAGTCG	TGATTGTAACCCAAGTAGCTGG
Mof	ACCTCAAAAGTGCCCAGTATAAG	AAACCCAGATCCCAAGCAG
Hdac1	GAGATGACCAAGTACCACAGTG	AAACAAGCCATCAAACACCG
Hdac2	TGACAAACCAGAACACTCCAG	TCTCCATCCTCATCTCCACTG
Kdm2a	AAGCCCGACGCATGAACAATAAGC	TGCCAAGTCCATCGTAATCCAGGT
Kdm8	TGCACACACCTTACTCAGGGTGAA	TTCAGCCAATGAGCTTCCCTTCCT
Setd3	AAGTGACAGGCTCTACGCCATGAA	AGTCTCTTTCAGCTCCTCTTCGGT
H1a	CATCACCACCAAGGTGTCAG	TTGGAAACTGCAGGCTTCTT
H1b	GTAGAGAAGTCTCCCGCCAAG	CCTTAGTGATGAGCTCGGACA
H1c	AAGGTCAAGAGCGCGTCTAA	GGGAGGCAGCCTACTTTTTC
H1d	ACGGCCAAGAAGACTCCGAAGAA	TGGGCTTAGCAGCCTTCACCTTC
H1e	AGCTAAGAGCCCGAAGAAGG	AGGCTTGGAGGTTTTTGGTT