LETTERS

A histone H3 methyltransferase controls epigenetic events required for meiotic prophase

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Epigenetic modifications of histones regulate gene expression and chromatin structure[1,2]. Here we show that Meisetz (meiosis-induced factor containing a PR/SET domain and zinc-finger motif) is a histone methyltransferase that is important for the progression of early meiotic prophase. *Meisetz* transcripts are detected only in germ cells entering meiotic prophase in female fetal gonads and in postnatal testis. Notably, Meisetz has catalytic activity for trimethylation, but not mono- or dimethylation, of lysine 4 of histone H3, and a transactivation activity that depends on its methylation activity. Mice in which the *Meisetz* gene is disrupted show sterility in both sexes due to severe impairment of the double-stranded break repair pathway, deficient pairing of homologous chromosomes and impaired sex body formation. In *Meisetz*-deficient testis, trimethylation of lysine 4 of histone H3 is attenuated and meiotic gene transcription is altered. These findings indicate that meiosis-specific epigenetic events in mammals are crucial for proper meiotic progression.

In sexual reproduction, meiosis reduces the ploidy of the genome and generates genomic diversity by shuffling information between homologous chromosomes. To accomplish meiosis, the transcription of several meiotic genes must be properly orchestrated over time as meiosis progresses. Transcriptional control of gene expression depends crucially on DNA accessibility, which is epigenetically regulated by histone modification[1,2]. The methylation of lysine 4 of histone H3 (H3K4 methylation) is a well-characterized feature of transcriptionally active genes[3–6], indicating that the action of histone methyltransferase (HMTase) on H3K4 marks genes for transcriptional activation according to specific tissue and temporal patterns. Although HMTases that catalyse H3K4 methylation have been identified in mammals[7–12], it remains unclear how the epigenetic modification is regulated during meiosis.

To elucidate transcriptional factors controlling the initiation and progression of meiosis, we identified genes whose expression was increased during entry into meiosis by subtracting complementary DNAs of mitotic primordial germ cells at embryonic day 11.5 (E11.5) from those of meiotic female primordial germ cells at E13.5. Of the genes identified (Supplementary Fig. S1), one encoded a putative transcription factor that we named Meisetz. The deduced amino acid sequence of Meisetz has a PR/SET domain (the catalytic domain of HMTases) in its amino-terminal portion and a C2H2-type zinc-finger

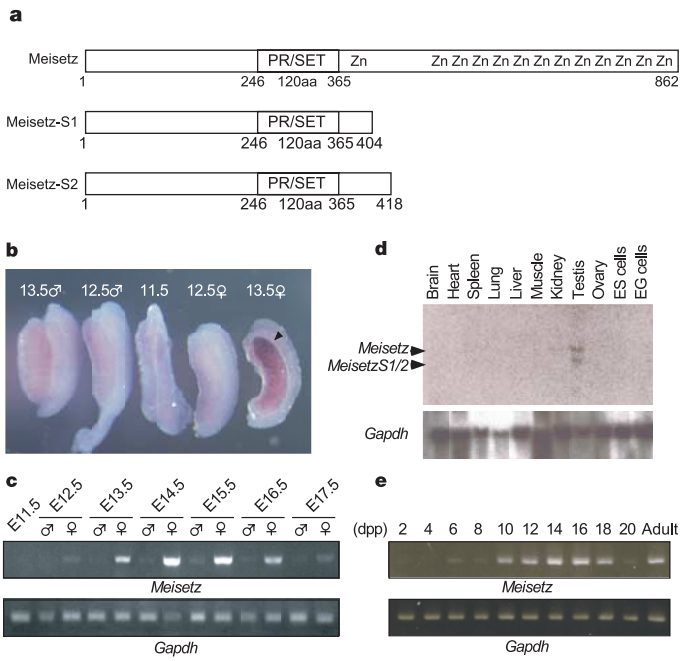

Figure 1 | Molecular structure and expression of the *Meisetz* gene. a, Domains in the deduced sequences of Meisetz protein and its short isoforms. Numbers indicate the amino acid sequence positions of each domain. **b**, *In situ* hybridization analysis of *Meisetz* expression in fetal gonads. *Meisetz* transcripts were detected in only E13.5 female gonad (arrowhead). **c**, RT–PCR analysis of *Meisetz* expression in fetal gonads. cDNAs obtained from fetal gonads at the indicated developmental stages were amplified with primers for *Meisetz* and *Gapdh*. **d**, Northern blot analysis of *Meisetz* expression in adult tissues. *Gapdh* is shown as a control. **e**, RT–PCR analysis of *Meisetz* expression in the first round of spermatogenesis. cDNAs obtained from testis at the indicated days after birth were amplified with primers for *Meisetz* and *Gapdh*.

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motif in its carboxy-terminal portion (Fig. 1a and Supplementary Fig. S2a). The amino acid sequence of the PR/SET domain of Meisetz is highly conserved in human (GenBank PRDM7, GenBank PRDM9) and rat (Ensembl ENSRNOG00000021493; Supplementary Fig. S2b). By rapid amplification of cloned ends (RACE), we found two shorter isoforms of the *Meisetz* gene lacking the zinc-finger motif (Fig. 1a and Supplementary Fig. S2a), which are generated by alternative splicing.

Transcripts of *Meisetz* were transiently increased in female gonads from E13.5 to E16.5 (Fig. 1b, c), the time during which meiosis proceeded from pre-meiotic replication to pachytene stages. Its expression was barely detectable in fetal male gonads. In adults, transcripts of *Meisetz* and its splicing variants were detected in testis, but not in any other tissue tested (Fig. 1d). Transcript abundance increased from 10 d post partum (dpp) to 18 dpp (Fig. 1e), during which time the first wave of spermatogenesis proceeds synchronously from pre-leptotene to pachytene stages[13]. The expression patterns of the two isoforms were identical to those of the full-length *Meisetz* gene in fetal gonads and adult testes (data not shown).

We tested whether Meisetz has HMTase activity by an *in vitro* methylation analysis. Recombinant glutathione *S*-transferase (GST)-fused Meisetz (GST–Meisetz) possessed HMTase activity that specifically catalysed methylation of native histone H3 (native H3) from calf thymus (Fig. 2a). To determine which residue is methylated by Meisetz, we tested the *in vitro* methylation of constructs in which GST was fused to the N terminus of histone H3 protein (GST–H3N) that are suitable for identifying the methylated lysine[14]. However, Meisetz did not methylate either GST–H3N or GST-fused full-length H3 (data not shown). Furthermore, Meisetz did not methylate these substrates after the GST tag was removed (data not shown). These results indicate that Meisetz might not catalyse the monomethylation of unmethylated recombinant histone H3, but might preferentially catalyse di- or/and trimethylation of native H3.

To evaluate this possibility, native H3 was incubated with GST–Meisetz and the methylated residues and their methylation status were determined by using methylation-specific antibodies. Unexpectedly, Meisetz showed catalytic activity for only H3K4 trimethylation (Fig. 2b, left). This trimethylation-specific activity of Meisetz was confirmed in experiments showing that Meisetz can restrictively methylate dimethylated H3K4 peptide (Supplementary Fig. S3). Furthermore, overexpression of *Meisetz* in COS7 cells also caused only H3K4 trimethylation (Fig. 2b, right), showing that the catalytic activity is exerted in physiological conditions.

Comparison of Meisetz with other molecules containing a PR/SET domain showed that this domain in Meisetz is distinct from those in established HMTases such as SET7 and SET9 (refs 7, 8), ALR1 (ref. 11), G9a (ref. 14), Suv39h1 and Suv39h2 (refs 15, 16) and SET1 (refs 17, 18; and data not shown). Notably, the PR/SET domain of Meisetz lacks the well-conserved sequence H/RxxNHxC, mutations of which abolish catalytic activity in known HMTases[15]; thus, histone methylation by Meisetz is accomplished by an unknown motif in the PR/SET domain.

To identify residues that are crucial for the HMTase activity, deleted or point-mutated variants of Meisetz and its isoform Meisetz-S1 were tested for methylation activity *in vitro*. On the basis of sequence alignment between Meisetz and other PR/SET-containing molecules, we identified Tyr 276 (Y276) and Gly 278 (G278) as potential catalytic residues. Deletion of ten amino acids including these residues abolished the enzymatic activity of Meisetz-S1 (Fig. 2c). Furthermore, replacement of G278, but not Y276, abolished the activity of both Meisetz-S1 and Meisetz (Fig. 2c), showing that G278 is central to the HMTase activity of Meisetz. Consistent with the fact that H3K4 trimethylation is a mark of transcriptionally active genes[4], wild-type Meisetz promoted transactivation but the replacement of G278 abolished this activity (Fig. 2d). Taken together, these results show that Meisetz possesses HMTase activity specific for H3K4 trimethylation and can activate transcription in a manner dependent on its HMTase activity. To our knowledge, Meisetz is the first tissue-specific HMTase identified in mammals; this tissue specificity, together with its specificity for H3K4 trimethylation, raises the possibility that Meisetz and structurally related HMTases (Supplementary Fig. S2b) regulate the expression of genes in restricted tissues and/or developmental stages.

To identify functions of Meisetz during meiosis, *Meisetz*$^{-/-}$ mice were generated by targeted disruption of the exon containing the initiation codon, which is shared in all isoforms (Supplementary Fig. S4a–c). No transcripts of either *Meisetz* or its splicing variants were detected in *Meisetz*$^{-/-}$ mice (Supplementary Fig. S4d). Mating of heterozygous (*Meisetz*$^{+/-}$) mice gave rise to offspring in the expected mendelian distribution (data not shown). *Meisetz*$^{-/-}$ mice were viable and showed no significant alteration in body weight

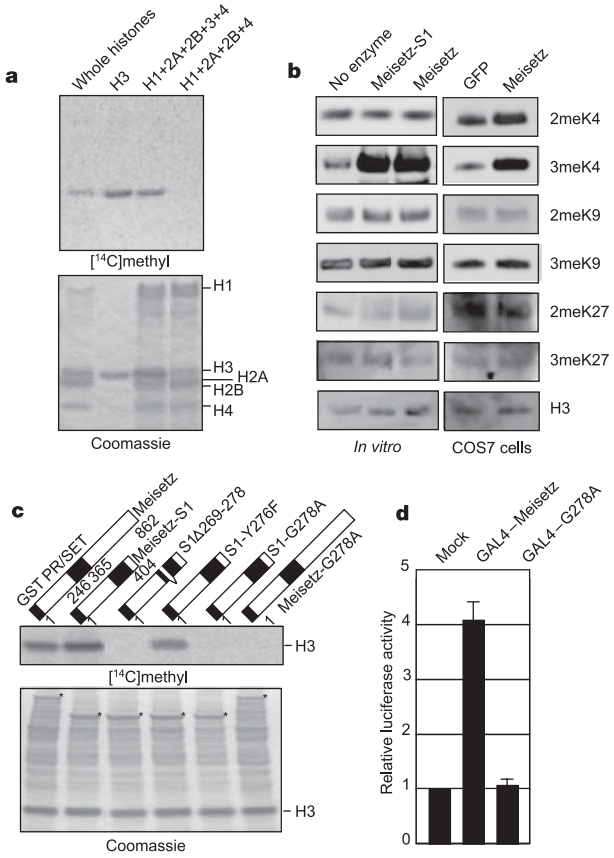

Figure 2 | Meisetz protein catalyses H3K4 trimethylation and activates transcription through its methylation activity. a, *In vitro* methylation of native histones by Meisetz. The indicated combinations of native histones were incubated with [^{14}C]*S*-adenosyl-L-methionine and GST–Meisetz, separated by SDS–PAGE, stained with Coomassie (bottom), and visualized by autoradiography (top). **b**, Western blot analysis to determine the lysine residue methylated by Meisetz. Native H3 incubated with *S*-adenosyl-L-methionine and GST–Meisetz or GST–Meisetz-S1 (left), and whole-cell lysates from COS7 cells in which Meisetz or GFP were transiently overexpressed (right), were subjected to western blotting with specific antibodies. **c**, Identification of a key residue for HMTase activity of Meisetz. Native H3 was incubated with [^{14}C]*S*-adenosyl-L-methionine and the indicated deletion or point-mutation variants of GST–Meisetz, separated, stained with Coomassie (bottom), and visualized by autoradiography (top). Asterisks indicate the recombinant GST–Meisetz proteins used as enzyme. **d**, Transactivation activity depends on HMTase activity. Expression vector (GAL4–Meisetz or GAL4–G278A) or mock vector was transfected into COS7 cells together with a reporter vector and a control vector. Luciferase activities from the reporter vector were normalized to those from the control vector. Shown are luciferase activities relative to those obtained by transfection of mock vector. Error bars represent the s.d. from three independent experiments.

or appearance (data not shown); however, both sexes of *Meisetz*$^{-/-}$ mice were sterile.

The weight of testes in 8-week-old *Meisetz*$^{-/-}$ mice was nearly 75% less than that of wild type (wild type, 115.3 ± 7.8 mg; *Meisetz*$^{-/-}$, 29.0 ± 2.2 mg). In 8-week-old *Meisetz*$^{-/-}$ testes, there were no round spermatids, elongated spermatids or spermatozoa (Fig. 3a, b). Using immunofluorescence staining with TRA369 monoclonal antibody, which specifically reacts with both pachytene spermatocytes and spermatids[19], and with a polyclonal antibody against SCP3, a lateral component of synaptonemal complex, we confirmed the accumulation of TRA369^{+} SCP3^{+} pachytene spermatocytes but not TRA369^{+} SCP3^{-} spermatids in *Meisetz*$^{-/-}$ testis (Fig. 3c, d). In *Meisetz*$^{-/-}$ females, there were no growing follicles at any developmental stage in 5-week-old ovaries (Fig. 3e, f), and few germ cells were recognized in neonatal ovaries (Fig. 3i, j). The number of germ cells in gonads decreased from E17.5 onwards (Fig. 3g, h) when meiosis was proceeding through the pachytene stage. These findings show that gametogenesis was disrupted at the pachytene stage in both sexes of *Meisetz*$^{-/-}$ mice.

We examined details of meiotic deficiencies in *Meisetz*$^{-/-}$ testis in spread cells. At the leptotene stage, the distribution of histone H2AX phosphorylated on serine (termed γH2AX), which marks sites of double-stranded breaks (DSBs)[20], showed no significant difference between wild-type and *Meisetz*$^{-/-}$ spermatocytes (Fig. 3k, l). At zygotene and pachytene stages, however, γH2AX was inappropriately retained on each chromosome in *Meisetz*$^{-/-}$ spermatocytes (Fig. 3n, p), whereas it disappeared from autosomal regions of synapsed chromosomes and converged around the sex chromosomes to form the sex body in wild type[20] (Fig. 3m, o). Similar to spermatocytes, female fetal germ cells at the pachytene stage showed more γH2AX in *Meisetz*$^{-/-}$ than in wild-type mice (Fig. 3q, r). In addition to abnormal retention of γH2AX, the pachytene chromosomes of *Meisetz*$^{-/-}$ mice were frequently branched and connected with other chromosomes (Fig. 3p, r), indicating that pairing between homologous chromosomes was also impaired in *Meisetz*$^{-/-}$ mice.

DMC1 protein, which is involved in recombination-associated DNA repair in meiosis[21,22], was poorly localized in γH2AX-stained regions in *Meisetz*$^{-/-}$ spermatocytes (Fig. 3t), whereas in wild-type spermatocytes most of the DMC1 foci were colocalized with γH2AX signals (Fig. 3s). In addition, the number of DMC1 foci in *Meisetz*$^{-/-}$ spermatocytes was less than half of that in wild type (Fig. 3t, s, and data not shown). Because the level of *Dmc1* transcripts in *Meisetz*$^{-/-}$ testis was comparable to that in wild type (data not shown), DMC1 foci may not be stable unless they properly localize at DSB sites[23,24]. These results suggest that DSB repair cannot proceed properly in *Meisetz*$^{-/-}$ spermatocytes due to failed localization of DMC1 at DSB sites. Thus, we conclude that meiotic arrest in *Meisetz*$^{-/-}$ spermatocytes is caused by impairment of DSB repair and consequent impairment of pairing between homologous chromosome and sex body formation.

To address further the functional requirement of Meisetz in meiosis, we examined the status of H3K4 trimethylation by immunofluorescence in juvenile testes at 14 dpp, when the first wave of spermatogenesis synchronously proceeds to early pachytene. There were no histological differences between wild-type and *Meisetz*$^{-/-}$ testes at this age (data not shown). Signals for H3K4 trimethylation were increased in pachytene spermatocytes in wild-type testis, but this increase was reduced in *Meisetz*$^{-/-}$ testis (Fig. 4a, b). The amount of H3K4 trimethylation in 14-dpp *Dmc1*$^{-/-}$ testes, in which spermatogenesis is arrested at the pachytene stage owing to impairment of chromosomal pairing[21,22], was similar to that in wild type (Fig. 4a, b), excluding the possibility that the attenuated H3K4 trimethylation in *Meisetz*$^{-/-}$ testis is a consequence of impaired chromosomal pairing followed by transcriptional repression[25,26]. The weak increase in H3K4 trimethylation in *Meisetz*$^{-/-}$ pachytene spermatocytes as compared with somatic cells indicates that an

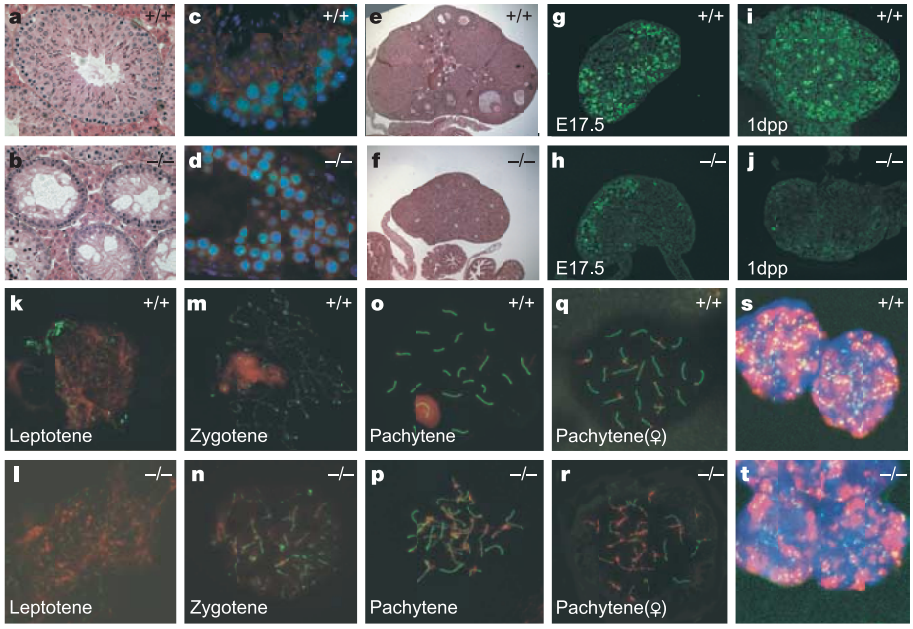

Figure 3 | Impairment of meiotic progression in *Meisetz*$^{-/-}$ mice. Haematoxylin and eosin (HE)-stained sections of wild-type (**a**) and *Meisetz*$^{-/-}$ (**b**) testis showed arrest of spermatogenesis in the *Meisetz*$^{-/-}$ testis. Sections of wild-type (**c**) and *Meisetz*$^{-/-}$ (**d**) testis stained by anti-SCP3 antibody (green), TRA369 monoclonal antibody (red) and DAPI (blue) confirmed arrest of spermatogenesis at pachytene. HE-stained sections of wild-type (**e**) and *Meisetz*$^{-/-}$ (**f**) ovaries, and anti-mVASA (green) immunofluorescence of sections of wild-type (**g**, **i**) and *Meisetz*$^{-/-}$ (**h**, **j**) ovaries showed arrest of oogenesis in *Meisetz*$^{-/-}$ ovary. The developmental stages of the ovary are indicated in each panel. Testicular (**k**–**p**) and ovarian (**q**, **r**) cells were spread and then stained with anti-SCP3 (green) and anti-γH2AX antibody (red). The developmental stages of meiotic prophase based on SCP3 kinetics are indicated in each panel. Wild-type (**s**) and *Meisetz*$^{-/-}$ (**t**) testicular cells were spread and then stained with anti-DMC1 (green) and anti-γH2AX (red) antibodies and DAPI (blue).

unidentified HMTase also functions in trimethylation. In contrast to H3K4 trimethylation, the amount of H3K4 dimethylation was higher in *Meisetz*$^{-/-}$ testis than in wild type, indicating that dimethylated H3K4 accumulates in *Meisetz*$^{-/-}$ spermatocytes owing to disruption of the H3K4 trimethylation reaction. Taken together, these results indicate that Meisetz has essential functions in spermatocytes through its H3K4 trimethylation of a portion of a genome; this H3K4 trimethylation may activate transcription of a group of genes that are crucial for the progression of meiotic prophase.

To address the possibility that an alteration in gene expression is responsible for the meiotic defects in *Meisetz*$^{-/-}$ mice, we screened *Meisetz*$^{-/-}$ mice for genes showing reduced expression by microarray and polymerase chain reaction with reverse transcription (RT–PCR) using cDNA from 14-dpp testes (data not shown). Of the genes identified, one candidate, *4932411A10Rik* (NCBI accession NM177719), was investigated further because its predicted amino acid sequence has a GHKL ATPase motif in its N-terminal region, a motif that is well conserved among DNA mismatch repair, topoisomerase, histidine kinase and HSP90 proteins[27] (Supplementary Fig. S5). *4932411A10Rik* transcripts were barely detected in *Meisetz*$^{-/-}$ testis (Fig. 4c), but were expressed in *Dmc1*$^{-/-}$ testis, indicating that the attenuated transcription was caused by loss of Meisetz but not by meiotic arrest. H3K4 trimethylation on the transcription start site of the gene was attenuated (Fig. 4d). In addition, *4932411A10Rik* transcripts were detected only in testis among the adult tissues tested (Fig. 4e) and were transiently expressed from pre-meiotic replication to the early pachytene stage (Fig. 4f, g), corresponding with the time of *Meisetz* expression. Taken together, the results strongly suggest that the *4932411A10Rik* gene is directly regulated by Meisetz and is involved in early meiotic progression.

Here we have identified a meiosis-induced histone trimethyltransferase, Meisetz, and have shown that it is essential for progression through early meiotic prophase, including DSB repair, homologous chromosome pairing and sex body formation. We propose that Meisetz might regulate gene expression through meiosis. Another study has provided evidence that an unknown HMTase for H3K9 also has a role in spermatocytes[28]. Our results also suggest that an additional HMTase trimethylates H3K4 in pachytene spermatocytes. These facts lead to the possibility that there is a previously unknown group of meiosis-specific HMTases responsible for the proper control of both meiotic gene expression and chromosomal structure, and we propose that *Meisetz* is the first example of such a group of molecules. We plan further studies to clarify how the genome-wide alternation of histone modifications, including trimethylation by Meisetz, contributes to the structural control of chromosomal alignment in mammalian meiosis.

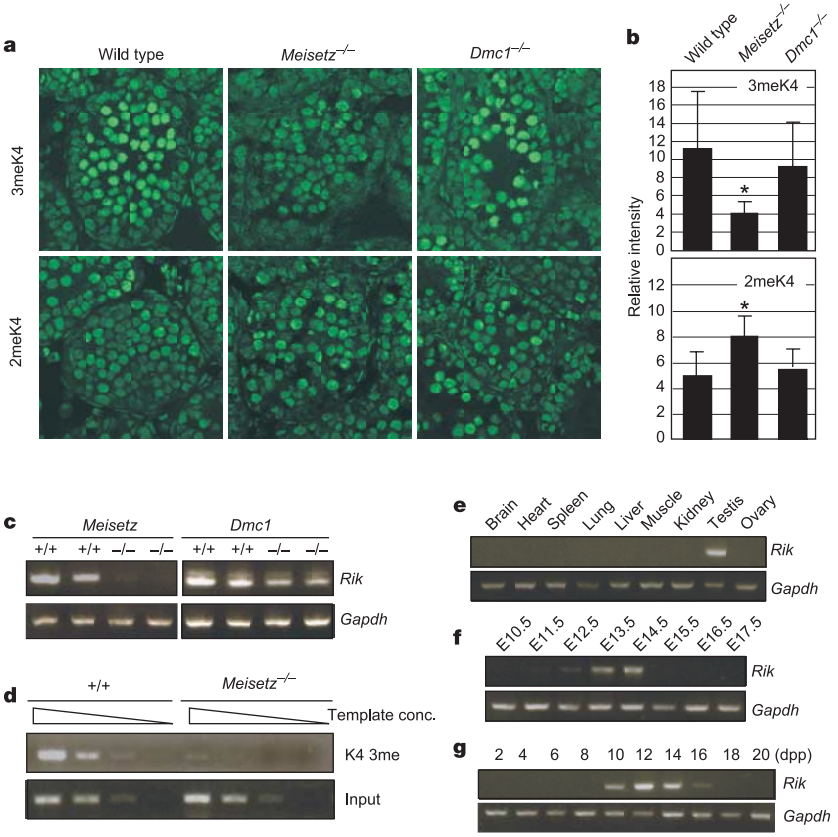

Figure 4 | Downregulation of H3K4 trimethylation and meiotic gene expression in *Meisetz*$^{-/-}$ testis. **a**, Immunofluorescence analysis of H3K4 trimethylation and dimethylation in 14-dpp testis. Sectioned testes of the indicated genotypes were stained with antibodies against trimethylated (3meK4) or dimethylated (2meK4) H3K4. **b**, Relative signal intensity of H3K4 trimethylation and dimethylation. Quantification of the relative intensity of fluorescence signals is described in Supplementary Methods. Asterisks indicate that the difference in signal intensity between *Meisetz*$^{-/-}$ and wild type was statistically significant ($P < 0.05$; Welch's *t*-test). Error bars indicate the s.d. **c**, Expression of the *4932411A10Rik* gene in *Meisetz*$^{-/-}$ testis. Transcripts of *4932411A10Rik* (*Rik*) or *Gapdh* were amplified by using cDNAs from 14-dpp testes of the indicated genotypes. **d**, Chromatin immunoprecipitation analysis of a putative transcriptional start site of the *4932411A10Rik* gene. DNA precipitated with antibodies against trimethylated H3K4 (K4 3me) or input DNA derived from testicular cells of each genotype was serially diluted and used for PCR. Additional PCR analyses using three *Meisetz*$^{-/-}$ and two wild-type (+/+) littermates gave similar results (not shown). **e**–**g**, Meiosis-specific expression of the *4932411A10Rik* gene. Transcripts of *4932411A10Rik* were amplified using cDNA from adult tissues (**e**), fetal female gonads (**f**) and juvenile testes (**g**). Tissues and developmental stages tested are indicated in each panel.

METHODS

Detailed methods are given in the Supplementary Information.

Vectors and methylation and transactivation assays. Full-length and short isoforms of *Meisetz* cDNA were amplified from testis cDNA of C57BL/6N mice by PCR. We amplified the genomic fragments for a targeting vector from the genomic DNA of E14 ES cells. *In vitro* methylation assays were done by using native histones or H3K4 peptides as substrates and GST–fused Meisetz as enzymes. For the GAL4 transactivation assay, GAL4–Meisetz, GAL4–G278A or mock vector, pFR–luc and phRL–tk were transfected into COS7 cells, and after cultivation cells were lysed and the luciferase activity of each was determined.

Tissue analysis, RT–PCR and chromatin immunoprecipitation. For immunofluorescence analyses of tissues, testes or ovaries were fixed in 4% paraformaldehyde overnight, embedded and sectioned. For RT–PCR analysis, total RNAs from whole gonads of various developmental stages were used for cDNA synthesis by using oligo d(T) primer. Chromatin immunoprecipitation assay was done with dispersed testicular cells. Cells were fixed and sonicated, and the lysate was reacted with rabbit antibodies against trimethylated H3K4. DNA was extracted from the precipitated nucleosomes and subjected to PCR analysis.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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