CENP-V is required for proper chromosome segregation through interaction with spindle microtubules in mouse oocytes

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Proper chromosome segregation is essential to avoid aneuploidy, yet this process fails with increasing age in mammalian oocytes. Here we report a role for the scarcely described protein CENP-V in oocyte spindle formation and chromosome segregation. We show that depending on the oocyte maturation state, CENP-V localizes to centromeres, to microtubule organizing centers, and to spindle microtubules. We find that Cenp-V−/− oocytes feature severe deficiencies, including metaphase I arrest, strongly reduced polar body extrusion, increased numbers of mis-aligned chromosomes and aneuploidy, multipolar spindles, unfocused spindle poles and loss of kinetochore spindle fibres. We also show that CENP-V protein binds, diffuses along, and bundles microtubules in vitro. The spindle assembly checkpoint arrests about half of metaphase I Cenp-V−/− oocytes from young adults only. This finding suggests checkpoint weakening in ageing oocytes, which mature despite carrying mis-aligned chromosomes. Thus, CENP-V is a microtubule bundling protein crucial to faithful oocyte meiosis, and Cenp-V−/− oocytes reveal age-dependent weakening of the spindle assembly checkpoint.

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Mammalian oogenesis is burdened with comparably high rates of failures that may result in chromosome mis-segregation and aneuploidy (for reviews see1–9). These errors happen during oocyte meiosis, which includes a very prolonged period of arrest, and increase with advancing age. Several mechanisms were proposed to contribute to this problem. One prominent mechanism is a gradual deterioration of cohesion (for reviews see8,10–12), which ought to maintain sister chromatid cohesion throughout oocyte lifespan. Cohesion is loaded onto oocyte chromosomes at the entry into meiosis with no or very little replenishment later. The increasing loss of sister chromatid cohesion with consequently loss of chiasmata in meiosis I and weakening of sister centromere cohesion up to anaphase II is considered a major factor in age-dependent oocyte chromosome mis-segregation. Several other mechanisms likely also contribute to oocyte ageing and problems associated with it (for reviews see9,13–19). Telomere damage also increases with progressing age and may cause chromosome end fusions and thus aberrant chromosome segregation. Likewise, erroneous attachment of microtubules to the kinetochores may lead to mis-segregation if not corrected during a spindle assembly checkpoint (SAC) mediated arrest. It appears as if the control mechanisms provided by the SAC also weaken with advancing age although this has not yet been clearly demonstrated. Thus, for persistent maintenance of oocyte chromosome integrity and proper segregation a well-balanced system of monitoring, error correction, and strict control pathways are required. This, however, is not fully sufficient as it fails in the long term. In this communication we describe an additional factor that plays a central role in the control of oocyte meiosis, CENP-V.

Human CENP-V is a 275 amino acid protein described so far in only very few publications, initially by William Earnshaw in 2008. This group identified it as a kinetochore component in proteome analysis of human mitotic chromosomes20. The 29.7 kDa protein CENP-V (pI 9.8) is highly conserved among vertebrates. In HeLa cells, it localizes to the mitotic kinetochores from prometaphase to metaphase and moves to the spindle mid-zone in anaphase. Cysteines within CENP-V may coordinate Zn++ which possibly mediates DNA binding. When overexpressed in HeLa cells, CENP-V causes hypercondensation of pericentromeric heterochromatin. Knock-down of CENP-V in HeLa cells leads to decompaction of chromosomes, decondensation of the heterochromatin. Metaphase alignment of chromosomes in CENP-V depleted HeLa cells is impaired and lagging chromosomes appear in anaphase20. Out of the context of mitosis, CENP-V appears to be involved in the regulation of microtubule structures as it promotes cell migration by targeting Src family kinases to the microtubule network in the leading edge of migrating cells21, CENP-V also supports tubulin acetylation at the basal bodies and axonemes of primary cilia22 – a posttranslational tubulin modification that is thought to enhance the resilience of microtubules to mechanical stress23.

In this work, we investigated the role of CENP-V in oocytes as their particularly delicate meiotic and spindle features may require functional CENP-V during maturation. We describe CENP-V as an important regulator of oocyte microtubule-kinetochore associations and chromosome segregation, whose absence causes age-dependent failure of the SAC and aneuploidy.

Results

CENP-V changes its localization during meiosis in mouse oocytes. CENP-V has been described as a component of the kinetochore from prometaphase to metaphase during mitosis20. To test whether this localisation is conserved in meiotic prophase I we stained mouse embryonic oocyte spreads with an anti-CENP-V antibody (Fig. 1a) raised against the full-length mouse protein and validated on CENP-V deficient cells (see next section). Unless otherwise specified, all experiments described below were done using young adults, i.e. 6 weeks to 3 months old mice. In wild-type (wt) mice we observed CENP-V to appear in pachytene yielding a diffuse signal. At the end of meiotic prophase in diakinesis CENP-V was found in the vicinity of centromeres and the surrounding heterochromatin as observed by co-staining with the centromere marker ACA (Fig. 1a). This pattern is similar to that seen in spermatoocyte prophase I, where CENP-V localized to the centromere region and external to the centromere protein CENP-A from the pachytene stage onwards, similar to what has been described for mitotic HeLa cells20 (Sup. Figure 1). However, CENP-V was excluded from the spermatoocyte X/Y chromosomes, the sex body chromatin.

The distribution of CENP-V during meiotic resumption was analyzed in mouse oocytes at different stages of maturation. To allow fixed and live-cell imaging, oocytes expressing CENP-V-GFP and H2B-RFP after injection of Cenp-V-GFP and H2B-RFP mRNA at the arrested germinal vesicle (GV) stage were used (Fig. 1b). At the stage of germinal vesicle breakdown (GVBD), the CENP-V-GFP signal covered the entire chromosomes. Since Honda et al. suggested an association of CENP-V with microtubules21, some GVBD oocytes were fixed and probed with an anti y-tubulin antibody as a marker for microtubule organising centres (MTOCs). Metaphase I and II spindles were stained with SiR Tubulin (Fig.1b and supplementary mov. S1). During meiotic resumption we observed three different pools of CENP-V: (i) at the chromosomes, (ii) at the MTOCs (Fig. 1b, upper row), and (iii) at the meiotic spindles. At metaphase I CENP-V is mainly enriched at the spindle and this was maintained throughout metaphase II (Fig. 1b, middle and lower rows). To get an unbiased quantification of the localization of CENP-V to the spindle, we measured the Pearson’s coefficient, using an imaging algorithm to measure the colocalization along z-stacks between CENP-V-GFP and SiR Tubulin. Notably, in the spindle pole Pearson’s coefficient was close to 1, indicating an almost complete colocalization. On the contrary, colocalization of CENP-V and SiR Tubulin decreased towards the spindle center, suggesting that CENP-V does not show a homogenous distribution in the spindle but rather accumulates at the pole (Fig. 1c). The same pattern was observed by staining fixed oocytes with an anti-CENP-V antibody (Fig. 2a, Sup. Figure 2).

This suggests that during female meiosis CENP-V expands its localization from around the centromere region in prophase to along metaphase I chromosomes, to the MTOCs, and to microtubules during metaphase I and PB extrusion (PBE).

Deletion of CENP-V arrests mouse oocytes at metaphase I. Would the lack of CENP-V affect oocytes maturation, meiotic progression? CENP-V homozygous null mice (Cenp-V/−/−) were generated by Cre recombinase-mediated deletion of the floxed exon 3 of Cenp-V, confirmed by DNA sequencing. We first assessed the loss of CENP-V in Cenp-V/−/− mice by immunofluorescence (IF) and western blot (WB) using commercial and in-house generated anti-CENP-V antibodies. The absence of CENP-V signals from chromosome spreads and from WB of Cenp-V/−/− oocytes or ovary confirmed loss of the protein, and these experiments also validated the antibodies (Fig. 2a, Sup. Figure 3a, b). Cenp-V/−/− mice were viable and females but not males were subfertile. Subfertility was observed when Cenp-V/−/− females were bred with wt, heterozygous, or homozygous CENP-V deficient males (Sup. Figure 4a). Because we observed subfertility only in Cenp-V/−/− females we focused on the role of CENP-V in oocyte meiosis.

Cenp-V+/- and Cenp-V/−/− oocytes were collected, maintained, and synchronized in prophase with intact germinal vesicles (GVs) by incubation in milrinone-containing medium. Two hours after removal of milrinone Cenp-V+/- and Cenp-V/−/− oocytes...
underwent germinal vesicle breakdown (GVBD), i.e. meiotic resumption. In average, Cenp-V−/− oocytes required significantly longer to undergo germinal vesicle breakdown (GVBD) (Fig. 2b; Cenp-V+/+: 83.7% ± 16.18 at 2.5 h; Cenp-V−/−: 54% ± 15.33 at 2.5 h). Twenty hours after the GV stage, 74.3% ± 6.24 of Cenp-V+/+ but only 41.6% ± 7.95 of Cenp-V−/− oocytes have extruded the first polar body (PB). This process was also significantly slower in Cenp-V−/− oocytes than in Cenp-V+/+ oocytes (Fig. 2c). To test whether this reduction in PB extrusion was due to a specific role of CENP-V during meiosis and not due to an unspecific effect brought about by the absence of CENP-V from all tissues in the Cenp-V−/− mouse strain we performed similar experiments using
an enhancer-trap conditional deletion strain crossed with GDF9-iCre or SPO11-Cre. The oocyte-specific GDF9-iCre expresses Cre shortly after birth, i.e. in oocytes that have just reached dictyate arrest. The decrease in PBE in Cenp-Vfl/fl and Cenp-V+/+ mice was very similar to that seen in Cenp-V−/− oocytes confirming the specific role of CENP-V during oocyte meiosis (Fig. 2d, e). Similar results were also obtained using Vasa-Cre which is expressed in oocytes at around early zygote (not shown). Together this data suggests that CENP-V is not required in prophase I but is required after dictyate arrest.

To further check possible deleterious effects of CENP-V deficiency on oogenesis we quantified the number of GV cells per female and performed histological analysis of ovaries from Cenp-V+/+ and Cenp-V−/− mice. No differences were observed in GV cell numbers, and follicular development appeared to be normal as evaluated by hematoxylin/eosin staining (Fig. 2f, left two columns, Sup. Figure 3b). To assess a possible role of CENP-V during ageing we quantified the GV cell numbers and performed life imaging experiments in Cenp-V−/− females older than 12 months. We found a reduction of the GV cell number due to ageing but not to the absence of CENP-V (Fig. 2f).

Surprisingly, the reduction in PBE found in young Cenp-V−/− oocytes vanished in oocytes from old females (Fig. 2g). With the control steady at app. 78%, 44.7 ± 14.6% of the young the Cenp-V−/− oocytes underwent PBE compared to 71.7 ± 6.2% of old Cenp-V+/− oocytes. Assuming that the errors caused by the absence of CENP-V remain the same in young and old oocytes, this hints at the weakening of the key control mechanism, the spindle assembly checkpoint (SAC). Together, these results suggest that CENP-V plays an important role for faithful oocyte maturation and chromosome segregation. Indeed, we observed an increased frequency of MII oocytes harboring abnormal numbers of pairs of sister chromatids, i.e. aneuploidy (Fig. 2h, i). To assess aneuploidy in meiosis II oocytes we counted chromosomes, i.e. pairs of sister chromatids, on MII metaphase chromosome spreads. Mis-segregation leads to loss of chromosomes or increased numbers and thus any deviation from 20 pairs of sister chromatids is considered an aneuploidy. This analysis revealed almost 4-fold increased rates of aneuploidy in absence of CENP-V. Sister chromatid cohesion itself was not lost since we did not detect single, unpaired chromatids. The limited number of oocytes present in aged mice precluded an extensive analysis of aneuploidy in those, but when mice of 9, 16, and 21 months of age were analyzed (together 15 oocytes for littermate controls and 10 oocytes for Cenp-V−/−), we observed 46% of cells for the control and 60% of the Cenp-V−/− oocytes showing aneuploidy. Generally, aneuploidy increases with age, for example through cohesion decay. While due to low numbers the increase in aged Cenp-V−/− oocytes is only a trend, the data indicates a higher frequency of aneuploidy in aged CENP-V deficient oocytes which, however, contrary to young oocytes still proceed to the PB stage, consistent with the above data.

Chromosome alignment is erroneous and delayed in Cenp-V−/− oocytes. A failure to perform PBE could be triggered by chromosome mis-alignment which can lead to SAC activation and thus to arrest of oocytes before PBE. Since CENP-V accumulated at the centromeric region and at the meiotic spindle, both key structures for proper chromosome alignment and kinetochore capture, we decided to monitor chromosome alignment in the absence of CENP-V.

Oocytes proficient and deficient for CENP-V were analysed by time-lapse fluorescence imaging of microtubules (β-TUBULIN-GFP) and chromosomes (H2B-RFP) after injection of the respective mRNAs (Fig. 3 and supplementary mov. S2 and S3). The H2B-RFP signal was followed until the spindle reached the cortex prior to PBE (Fig. 3a). Ten hours after GV, 75% of the control cells showed full chromosome alignment at the metaphase plate compared to 40% of Cenp-V−/− cells (Fig. 3b). These values were very similar to the respective frequencies of PBE (Fig. 3c) and to the values of PBE in non-injected cells (Figs. 2, 3c), suggesting that chromosome mis-alignment contributes to the decrease in PBE and that injection per se had no effect. To test whether the lack of CENP-V and no other cause reduced PBE and chromosome mis-alignment we also injected Cenp-V-Gfp mRNA into Cenp-V−/− cells. Representative frames of the time course are shown in Fig. 3a, which includes an example of a Cenp-V−/− oocyte overexpressing β-TUBULIN-GFP/H2B-RFP that stayed arrested at MI and a Cenp-V−/− oocyte overexpressing CENP-V-GFP that successfully extruded its PB (supplementary mov. S3 compare to supplementary mov S4). Cenp-V−/− oocytes expressing CENP-V-GFP restored both, chromosome alignment and PBE to control values (Fig. 3b, c), confirming that the lack of CENP-V causes the chromosome mis-alignment that leads to the reduction of PBE. For clarity of the results we do not represent the percentage of PBE and chromosome alignment of the Cenp-V−/− oocytes expressing CENP-V-GFP as they behave like control oocytes (Fig. 1b, supplementary mov. S1).

Inhibition of Mps1 restores progression to metaphase II. The reduction in PBE in the absence of CENP-V correlates with chromosome misalignment, which is the likely cause of oocyte arrest at MI. Interestingly, we observed a decrease in PBE only in oocytes from young females, but not in oocytes from females 12 months or older. We speculated that the SAC increasingly fails to prevent anaphase in ageing oocytes carrying chromosome and/or spindle aberrations. An age-dependent decline in SAC stringency has been previously suggested. We hence tested if the SAC is responsible for the arrest of young Cenp-V−/− oocytes before PBE. Thus, we inhibited the SAC kinase Mps1 by adding reversine to the culture medium of Cenp-V−/− and Cenp-V+/+
oocytes at the GV stage (Fig. 4). PBE in reversine-treated control oocytes was accelerated and PBE in Cenp-V−/− cells was fully restored to control values. This suggests that the SAC arrests Cenp-V−/− oocytes of young females at metaphase I. Analysis of chromosome mis-alignment in reversine-treated oocytes from young adults revealed a significant increase. This indicates that upon SAC inhibition many more oocytes carrying misaligned chromosomes proceed to PBE and MII (Fig. 4b). Notably, unlike young Cenp-V−/− oocytes, aged Cenp-V−/− oocytes proceeded to PBE like controls (Fig. 2g). In the aged Cenp-V−/− oocytes a strongly increased frequency of cells carrying misaligned chromosomes was observed (Fig. 4b). The absence of MI arrest in aged oocytes despite this presence of mis-aligned chromosomes confirms that the SAC is not as active anymore in oocytes from...
Fig. 2 Cenp-V−/− oocytes are impaired in MI progression. a Expression of CENP-V protein in Cenp-V+/+ and Cenp-V−/− oocytes. CENP-V is probed by anti CENP-V antibody (green), DNA is stained by DAPI (blue). Scale bar = 5 μm. b Cumulative percentage of GVBD cells over time in Cenp-V+/+ and Cenp-V−/− oocytes. c Cumulative percentage of first polar body (PB) extrusion over time in Cenp-V+/+ and Cenp-V−/− oocytes. d Cumulative percentage of first polar body extrusion over time in Cenp-V+/+ Gdf9cre- and Cenp-V−/− Gdf9cre + oocytes. e Cumulative percentage of first polar body extrusion over time in Cenp-V+/+ Spot1cre- and Cenp-V−/− Spot1cre + oocytes. f Number of oocytes in young (<6 months) and old (>12 months) Cenp-V+/+ and Cenp-V−/− females as an average number of GV cells per female, and corresponding SD. g Percentage of first polar body (PB) extrusion after 20 h culture of oocytes from young (<6 months) and old (>12 months) Cenp-V+/+ and Cenp-V−/− females, shown as an average percentage per female. h Percentage of MI oocytes showing aneuploidy. i Examples of MI oocyte chromosome spreads of control and Cenp-V−/− oocytes. The numbers of chromosomes are indicated; size bar = 50 μm. Statistic: (b–h); data were expressed as mean ± SD and statistical differences were tested by a paired two tailed t-test (**p < 0.01, *p < 0.05). The exact p values are available in the source data file. T-test results between Cenp-V+/+ and Cenp-V−/− oocytes (c), Cenp-V+/+ Gdf9cre (d), Cenp-V+/+ Spot1cre (e) are not significant. i.e. they all showed the same phenotype. (b); statistical differences refer to the average of the data points between 1 and 5 h after GV. (c,d,e,f); statistical differences refer to the average of all data points. (b, c, f, g) n = 303 oocytes from 6 independent experiments; (d,e) n = 134 oocytes from 3 independent experiments and (i) n = 20 cells from 3 independent experiments.

Fig. 3 Chromosome alignment is reduced and delayed in Cenp-V−/− oocytes. Polar body extrusion and chromosome alignment return to control values upon Cenp-V-Gfp mRNA injection. a) Selected time frames from a time-lapse experiment showing progression through meiotic prophase until metaphase II in Cenp-V+/+ and Cenp-V−/− oocytes expressing β-TUBULIN-GFP (magenta), H2B-RFP (cyan, large image in grey) and/or CENP-V-GFP (green) after mRNA injection. At 09:30 h in metaphase I, the spindle is assembled and chromosomes are aligned in the equatorial plate (white dash line) in Cenp-V−/− oocyte. Note that Cenp-V−/− oocytes show normal chromosome alignment and extrude the PB only when expressing CENP-V-GFP. Oocytes were imaged after 4 h release from GV every 30 m. Maximum intensity projection is shown. See also supplementary Movies S2,S3,S4). Scale bar = 10 μm. b Percentage of cells that show chromosome alignment overtime of the experiment shown in (a). c Cumulative percentage of first polar body and chromosome alignment return to control values upon Cenp-V−/− oocytes injection. Note that at 10 h, 75% of control oocytes have reached full chromosome alignment compared to the 35% of Cenp-V−/− oocytes, and only the injection of Cenp-V-Gfp (green square line) mRNA restores the values to the control. c Cumulative percentage of first polar body extrusion overtime of the experiment shown in (a). Note that Cenp-V−/− oocytes properly extrude the PB only when expressing CENP-V-GFP (green square line) and not when expressing β-TUBULIN-GFP, H2B-RFP. See also supplementary movie S1. Statistic: (b, c) data were expressed as mean ± SD and statistical differences were tested by a two way Anova test (**p < 0.001, *p < 0.01, *p < 0.05). The exact p values are available in the source data file. Statistical differences refer to the average of all data points. Anova test between Cenp-V+/+ and Cenp-V−/− injected was not significant. Anova test between Cenp-V−/− and Cenp-V+/+ β-TUBULIN-GFP/H2B-RFP injected was not significant. n = 249 cells from 5 different experiments.

old females. Thus, CENP-V deficiency reveals age-dependent weakening of the SAC in oocytes.

CENP-V maintains spindle integrity in vivo and binds, diffuses along, and bundles microtubules in vitro. It has been suggested that the absence of CENP-V disturbs microtubule dynamics. A failure in microtubule dynamics could lead to problems in chromosome capture and alignment. Thus, we asked whether the lack of CENP-V affects oocyte spindle integrity. Cenp-V−/− oocytes were fixed and stained for microtubules at different time points; at 7 h after GV to study the recently formed M1 spindle...
and between 10-16 h to study the late MI spindle after arrest and after the spindle has reached the cortex. At late metaphase I, between 10-16 h of culture, 46% of Cenp-V$^{-/-}$ oocytes but virtually no control oocytes showed aberrant spindles suggesting that the lack of CENP-V indeed disrupts spindle integrity (examples are shown in Fig. 4c and Sup. Figure 5a). In those cells that still show a defined spindle shape, the spindle pole width was measured. We observed that lack of CENP-V leads to loss of the canonical barrel-shaped spindles as the poles are less defined, diffusely widened, in Cenp-V$^{-/-}$ oocytes (Sup. Figure 5b).

As previous work suggests that CENP-V can bind microtubules on its own 22, we hypothesised that CENP-V-microtubule interactions might directly stabilise the acentrosomal meiotic spindle. To test this hypothesis, we expressed recombinant
His₆-CENP-V-eGFP in insect cells and purified the fusion protein by immobilised metal affinity and size exclusion chromatography (Supp. Figure 6). Biophysical properties of CENP-V were studied in vitro. We first quantified the microtubule-binding capacity of CENP-V-eGFP, by titrating increasing amounts of protein onto surface-bound, taxol-stabilised ATTO 647N-labelled microtubules in 80 mM PIPES, pH 6.8, 1 mM MgCl₂, 1 mM EGTA (BRB80) and visualising the interaction by total internal reflection fluorescence (TIRF) microscopy (Fig. 5a, Sup. Figure 7A, B and Sup. Mov. S5). The binding of CENP-V-eGFP to the microtubule lattice was quantified by calculating the average GFP-intensity per micrometer for at least 100 microtubules per condition for three independent technical replicates. The resulting binding saturation curve was best fitted with a cooperative binding (Hill) model,
suggesting that binding of CENP-V to the microtubule lattice is cooperative (Fig. 5b). Binding of CENP-V-eGFP occurred with a $K_d$ of $53.2 \pm 4.5$ nM (Fig. 5b), while single microtubule-associated particles were already evident at 1 nM (Fig. 5a–c).

To characterise the lattice behaviour of single CENP-V-eGFP particles, we next recorded three-minute time-lapse movies at 100 milliseconds time resolution, visualising the movement of 1 nM CENP-V-eGFP in BRB80 at 25 °C on surface-bound, taxol-stabilised ATTO 647N-labelled microtubules by TIRF microscopy. In general, CENP-V-eGFP particles stayed attached to the microtubule lattice, with only a few associations or dissociation events to be observed within up to fifteen minutes (Fig. 5c, supplementary Mov. S5, supplementary Fig. 8A, B). Lattice-bound CENP-V particles can be forced to dissociate from the microtubule by an excess of free tubulin - but not by BSA - indicating that the CENP-V-tubulin interaction is specific (Sup. Fig. 8C). Particles showed complex motility on the microtubule lattice that can be described either by i) continuous, rather “fast” diffusion (Fig. 5c I, II), ii) very slow diffusion (Fig. 5c III, IV) with interdispersed short but fast diffusing episodes (Fig. 5c, white arrowheads) or iii) a mixture thereof, where CENP-V particles switch between modes. Furthermore, motile particles eventually became stationary for extended periods on the microtubule lattice (Fig. 5c IV, blue arrowhead) to end of kymograph, switch motile to stationary and Fig. 5c V, beginning of kymograph to blue arrowhead, switch stationary to motile) or remained associated with the microtubule end (Sup. Fig. 8A). At microtubule intersection, CENP-V-eGFP particles occasionally switched microtubule tracks and explored both microtubules (Sup. Fig. 8D). A mean squared displacement analysis including all CENP-V-eGFP particles that showed some episodes of movement (excluding stationary episodes at microtubule ends or microtubule intersections) during the duration of the time-lapse movie, produced a rather low apparent diffusion coefficient of $D = 0.0192 \pm 0.0006 \mu m^2 s^{-1}$ ($n = 136$) (Fig. 5d). As expected from the complex CENP-V motility on the microtubule lattice (Fig. 5c), however, the diffusion coefficient of single CENP-V-eGFP particles are distributed over at least one order of magnitude with maximum values of $D = 0.15 \mu m^2 s^{-1}$ (supplementary Fig. 8E).

Interestingly, motile CENP-V-eGFP particles also showed a rather broad intensity distribution with four local maxima at $131 \pm 4$, $259 \pm 4$, $432 \pm 11$ and $760 \pm 79$ arbitrary units (a.u., $n = 249$) (Fig. 5e). A comparison with the average intensity loss of $118 \pm 8$ a.u. ($n = 50$ bleaching steps from 28 particles) per GFP during bleaching events of these particles suggests that - in line with the observed cooperative binding behaviour - microtubule bound CENP-V can form multimers on the microtubule lattice (Fig. 5f, supplementary Fig. 9). Hence, we hypothesised that the broad distribution of diffusion coefficients might be a direct cause of the inhomogeneous particle size distribution. In this case, we would expect larger multimeric particles to interact stronger with the microtubule lattice causing lower diffusion rates compared to smaller particles. Subsampling of the data by grouping the particles in 125 arbitrary unit bins, however, showed that the distribution of diffusion coefficients does not change significantly with increasing particle size (Supplementary Fig. 8F). This suggests that the broad distribution of diffusion coefficients observed for single CENP-V particles is caused by peptide properties other than multimerisation.

The cooperative binding behaviour of CENP-V and its capacity to multimerise (on the microtubule lattice) is reminiscent of other microtubule-associated proteins like PRC1/Ase1 that structurally reinforce the mitotic spindle by bundling interparallel microtubules in antiparallel overlaps of the spindle midzone12-36. We hence asked, whether also CENP-V has the capacity to drive microtubule bundle formation. To test this, we incubated taxol-stabilized ATTO 647N-labelled microtubules with 50 nM CENP-V-eGFP or non-GFP-tagged CENP-V for 15 min at room temperature, immobilized them on the surface of glass flow-cambers, and flushed out non-bound CENP-V prior to imaging. Indeed, both 50 nM CENP-V and CENP-V-eGFP induced the formation of large microtubule bundles that were absent from the buffer-only control (Fig. 5g).

In summary, we conclude that CENP-V is a bona fidea microtubule-associated protein that binds cooperatively to the microtubule. It remains attached to the microtubule for a prolonged period exploring it by diffusion and it forms multimers that are able to cross-link and bundle microtubules. It therefore shows all characteristics of a structural MAP like PRC1/Ase1. This could explain the loss of spindle integrity we observed in CENP-V deficient oocytes in vivo. Thus we measured microtubule bundling in the meiotic spindle (Sup. Fig. 10). To remove unstable microtubules, MI oocytes were cold-treated before fixation. Microtubules were visualised by anti-Tubulin-GFP antibody. The spindle was divided in five equal regions and the thickness of the most and least prominent microtubule in each region was measured and defined as a maximum and minimum level of bundling37. We observed a reduction in both, maximal and minimal microtubule thickness, i.e. bundling, in Cenp-V−/− oocytes (Sup. Fig. 10a). The maximum level of bundling in Cenp-V−/− oocyte spindles does not even reach the minimum grade of bundling in controls. The overall level of spindle fluorescence was also reduced in Cenp-V−/− cells compared to the control oocytes...
CENP-V is required for proper chromosome-microtubule attachment in metaphase I. It was suggested that the SAC control may be less stringent in oocytes than in somatic cells, not being able to recognize a single unattached kinetochore. It is uncertain whether the presence of one or two mis-aligned chromosomes would be sufficient to alone trigger the SAC. Failure to properly attach kinetochores to the spindle may add to the signals triggering the SAC. Therefore, we decided to assess chromosome-microtubule attachment in fixed cells by super-resolution microscopy to determine the number of unattached chromosomes in young and old oocytes at metaphase I and II. Before fixation, oocytes were incubated in a cold buffer to preserve stable, kinetochore-attached microtubules (K-fibres), while the less stable, non-attached microtubules are lost. This treatment allows us to visualise specifically those K-fibres that are well attached to the chromosomes. Because live imaging experiments (Figs. 2, 3) showed that in control cells at 10 h after GV almost all chromosomes are well aligned at the metaphase plate and the spindle is at the cortex but still ahead of anaphase and PBE, the attachments of homologous chromosomes were analysed in fixed oocytes after 8–10 h of maturation (Fig. 6). The levels of chromosome mis-attachment were similar to those in live cell experiments (Fig. 6a, compare to Fig. 3b), supporting our previous results and validating the time point of cell fixation. Again, a significantly higher number of Cenp-V+/− cells showed chromosome mis-attachment. Each oocyte was imaged as a z-stack with a spatial resolution of 145 nm allowing us to distinguish between single homologous pairs. At this high resolution we counted almost 3 mis-aligned chromosomes per Cenp-V+/− oocyte compared to less than 1 in controls (Fig. 6b and Supplementary Mov. S6 and supplementary Mov. S7).

In oocytes the absence of centrosomes is functionally replaced by self-organizing microtubule organising centres (MTOCs) that form de novo from a cytoplasmic microtubule network leading to the formation of the spindle and its polarity. Since we observed CENP-V colocalizing with MTOCs and with the spindle (Fig. 1 and Supplementary Fig. 2), β-TUBULIN staining was used to measure the spindle pole width (SPP width) as an indicator of spindle pole integrity (Fig. 6c, d). It is important to consider that in this experiment all oocytes are used for quantification although at 7–10 h of culture it is not possible to determine which oocytes will later extrude the PB and which will not. Further, at this time point one cannot predict which spindles become compromised. Therefore, although all values are represented in the box plot, only those values of Cenp-V−/+ bigger than the median value in Cenp-V+/+ are considered for statistic differences. We observed that Cenp-V−/+ spindles show increased variability in SPP width and often increased SPP width, i.e. there is a lack of spindle pole integrity in Cenp-type−/+ spindles. To discard the possibility that the observed phenotype was due to an unspecific effect of the cold treatment, the SPP width was also measured in non-cold treated cells yielding the same results (Sup. Fig. 5b). A central region at the equatorial plane was defined to quantify the K-fibres as arbitrary units of fluorescence of the tubulin channel within that region (Fig. 6c). Cold-treatment removed the unstable non-K-fibres. Cenp-V−/+ spindles showed a dramatic reduction in K-fibres: the control spindles showed 3-fold more K-fibres than the Cenp-V−/+ spindles (Fig. 6e).

Lastly we analysed the mode of microtubule attachment to the homologous chromosomes. Three different types of attachments were identified: (i) two sister centromeres attached by microtubules from different poles, i.e. the correct attachment to properly segregate the homologous chromosomes in anaphase I; (ii) one sister centromere attached by microtubules from one pole; and (iii) both sisters centromeres unattached. Indeed, we found entire homolog pairs unattached (selected examples are shown in Fig. 6f, Supplementary Mov S8, and Supplementary Mov. S9). About 30% of the homologous chromosomes in Cenp-V−/+ oocytes were unattached, while there were no unattached chromosomes in control cells. Of the remaining 70% of chromosomes, almost half showed a wrong attachment in absence of CENP-V with only one sister centromere attached. In control cells, only 15% wrong attachments were observed in total (Fig. 6g). We tested the possibility that wrong or entirely missing attachments are a consequence of a perturbed centromere structure, as described for HeLa cells where CENP-V was reduced. Therefore, sister centromere distances were measured in Cenp-V−/+ and Cenp-V+/+ metaphase I chromosomes spreads from non-cold treated, unperturbed cells, and no differences in centromere distance were found between both genetic backgrounds (Sup. Fig. 11). This indicates that sister centromere cohesion is not affected, consistent with our earlier results of lack of single unpaired chromatids in metaphase II.

Taken together these experiments show that CENP-V plays an important role in kinetochore-microtubule attachments and is required for proper metaphase I chromosome alignment and spindle formation.

CENP-V is required for correct chromosome-microtubule attachment during metaphase II. Since erroneous MT-chromosome attachments in metaphase II (MII) could lead to aneuploidy as well, we decided to take advantage of the fact that old Cenp-V−/+ oocytes progress to metaphase II. We performed cold treatment to visualize the attachments at MII in aged Cenp-V−/+ oocytes (>12 months). To be sure the cells have reached the MII arrest, we fixed the cells 5 h after PB extrusion as published, and performed quantifications as for MI (Fig. 7).

Like in MI, in MII we found a strong difference in chromosome alignment at the equatorial plate in Cenp-V−/+ oocytes: proper alignment in only 33% of the cells compared to 80% in control oocytes (Fig. 7a). The number of mis-aligned sister chromatids per cell was also higher in Cenp-V−/+ oocytes with more than 3 sister chromatids per cell, in some cells up to 5 mis-aligned sister chromatids were found (Fig. 7b). Following the same criteria as in the previous experiment (Fig. 6c) the K-fibers and the polarity of the spindle were quantified. In the absence of CENP-V there is a significant app. 2-fold reduction of K-fibers and a moderate loss of spindle polarity (Fig. 7c, d). As described in we found a high number of merotic or lateral attachments in old control and Cenp-V−/+ oocytes. However, we did not find any pair of sister chromatids unattached in controls compared to 23% of unattached sister chromatid pairs in Cenp-V−/+ oocytes (Fig. 7e, f). This is consistent with data shown in Fig. 2 on strongly increased aneuploidy in Cenp-V−/+ oocytes.

Thus, as in MI, CENP-V contributes importantly to MII spindle integrity, chromosome attachment, and chromosome alignment.

Discussion

Here we report that the poorly described protein CENP-V crucially contributes to mammalian meiosis I and II. In meiotic prophase I CENP-V accumulates at and near the centromeres. In oocytes, which feature a unique mode of spindle assembly through acen- trosomal MTOCs, CENP-V localises to these MTOCs and gradually
associates with the spindle when the oocytes mature, i.e. progress through meiosis I up to metaphase II. This spatial distribution is in line with data presented in the three papers describing CENP-V in somatic cells where it localises to the kinetochores, the mitotic spindle midzone, or other microtubule structures in the leading edge of migrating cells or primary cilia. The appearance of CENP-V starting in zygotene suggests that CENP-V, which is typically expressed in somatic cells and thus in premeiotic cells, vanishes at the entry into meiosis and is only later expressed again. The reason for this disappearance is unknown, but it indicates the rapid turnover of the protein.

Independently of oocyte age, the absence of CENP-V in oocytes results in aberrant spindle formation and in a significantly, up to a five-fold increased level of chromosome
misalignment with associated subfertility and aneuploidy. Several of the results such as meiotic resumption data were obtained not only from the constitutive CENP-V deficiency model but also from three germ cell-specific Cre-driven conditional CENP-V deficiencies demonstrating that the phenotypes observed are germ cell-intrinsic and not indirectly caused by deficiencies of surrounding somatic cells. CENP-V deficient spermatocytes however, did not display an obvious phenotype. As oocytes and spermatocytes assemble their spindle by different mechanisms, we hence propose that CENP-V is dispensable for the conventional centrosome-based mechanism used in spermatocytes. In contrast to spermatocytes, oocytes perform asymmetric cell division leading to the exclusion of the polar body. This process involves elaborate movement of the spindle to the cell cortex. In this context, CENP-V could render the spindle more resilient to mechanical stress during its relocation.

The resumption of oocyte meiosis and thus GVBD does not require CENP-V. This suggests that the preceding steps of early oogenesis do not depend on CENP-V. This hypothesis is supported by our observation of identical phenotypes in all CENP-V deficiency models, regardless of whether constitutive or induced in early prophase or shortly after birth, i.e. in dictyate arrested oocytes. To trigger the phenotypes described in this communication it is sufficient to induce CENP-V deficiency in these arrested oocytes and thus shortly before resumption of meiosis. For successful PBE, the chromosomes need to be all properly and k-12 bers become irregular and unstable shortly thereafter. Administering a SAC inhibitor allowed oocytes to perform PBE like the control indicating that the SAC is not involved in this process. We hence hypothesize that loss of CENP-V itself is not lost in this mutant, rather proper segregation of pairs of sister chromatids. We did not observe single, unpaired sister chromosomes in absence of CENP-V. Thus, centromeric sister cohesion itself is not lost in this mutant, rather proper segregation of pairs of sister chromatids is failing. When age-dependent cohesion loss...
happens its consequences are likely exacerbated by weakening of the SAC since a fully functional SAC would prevent oocytes that suffer cohesion loss from proceeding. Therefore, we suggest a cumulative effect of cohesion loss and SAC weakening as important drivers of age-dependent aneuploidy. This model does not exclude other mechanisms such as telomere damage-induced chromosome fusions that should also trigger the SAC, likely to fail in old oocytes.

Fig. 7 Metaphase II spindle and chromosome-microtubule attachments in oocytes fixed 5-7 h after polar body extrusion and cold treatment. (a) Percentage of cells showing chromosome misalignment. (b) Average number of chromosome misalignments per cell. (c) K-fiber quantification expressed as arbitrary units (A.U) of fluorescence (d) Spindle pole width (SPP width, w) quantification. (e) Example of chromosome-mt attachments in Cenp-V+/+ and Cenp-V−/− oocytes. A maximum projection of the whole spindle is shown for each genotype. Single planes of z-stacks are shown for every attachment. One example of a proper amphitelic attachment to the centromeres is shown for Cenp-V+/+ and Cenp-V−/− oocytes, indicated by a white dashed square. A merotelic or lateral attachment and three examples of unattached sister centromeres are shown for Cenp-V−/− oocytes, pink and yellow dashed square respectively. Microtubules are stained by anti β-tubulin and DNA stained by Hoechst 33258. The tip of the K-fiber attached to the sister centromere is indicated by a yellow arrow head. Centromeres are identified as the strongest region stained by Hoechst 33258. Images were taken on an Airyscan confocal microscope, the z plane distance was 145 nm. See also supplementary Movies S10 and S11. Scale bar = 10 µm (f) Chromosome-microtubule attachment quantification. Statistic: data were expressed as mean ± SD and statistical differences were tested by a paired two tailed T- test (**p < 0.01, ***p < 0.001, *p < 0.05). The exact p values are available in the source data file. Statistical differences refer to the average of all data points. (c,d) Data are represented as box plots where the middle line is the median, the lower and upper boxes correspond to the first and third quartiles and outlying points are plotted beyond the end of the whiskers. (a,b,c,d) n = 54 cells and (f) n = 79 attachments. All data come from 3 independent experiments.
The absence of CENP-V causes a failure to properly capture and align chromosomes and thus has a very significant biological consequence. Ageing CENP-V deficient oocytes, despite carrying abnormal numbers of chromosomes, underwent meiosis I up to metaphase II as the SAC failed. This contributes to either subfertility or to aneuploid embryos, some of which may survive, or both. Assuming that these basic mechanisms and processes are conserved between mouse and man, this is of obvious concern for human reproductive health.

**Methods**

**Isolation, culture and live imaging of mouse oocytes.** All mice were bred and maintained on a 12 h light/12 h dark cycle at 22 °C (± 2 °C) and 55 % humidity (± 5 %) in the animal facility of the medical faculty, Technische Universität Dresden, according to approved institutional guidelines. All animal experiments were performed according to the relevant ethical requirements and were approved by the state animal welfare office (Dr. B. Langen; license numbers TVA03/2016; TVVS1/2016; TVA07/2021).

In our study, we used 6–12 weeks old C57BL/6 females except for ageing studies in which 12–14 months old mice were used. Ovaries were isolated in M2 medium (Sigma) supplemented with 250 μM milrinone (Sigma) and punctured with a 27 gauge needle to release the oocytes. GV stage oocytes were collected with mouth pipettes (P200, Eppendorf) and prepared for immunostaining under paraffin oil (Sigma) at 37 °C. For analysis of GVBD and polar body extrusion oocytes were released into M2 medium and imaged every 30 min for up to 20 h on an inverted Nikon TE2000E microscope with a Plan APO 20 ×/0.75 NA objective. After the microtubule-binding assays, taxol-stabilised Atto647N-labelled microtubules were bound to the anti-α-tubulin antibody for 2 h before being washed twice with M2. The cells were washed at RT in 0.1% Tween 20 in PBS 3 x 5 min and mounted in Vectashield (H-1000; Vector Laboratories) plus 1 μg/ml DAPI (1:5–6-diamidino-2-phenylindolod) dye for cytoplasmic imaging. Fixed oocytes were imaged with an inverted Zeiss Axio Imager Z1 microscope using a 63 x/1.4 NA oil objective. Brightness and contrast of the images were modified for presentations.

**In vitro transfection, microinjection and oocyte live imaging.** β-tubulin-Gfp, histone H2b-Rfp, and Cenp-V-Gfp mRNAs were synthesized with the T3 or T7 Message mMachine Kit (Ambion) according to the manufacturer’s instructions and purified using RNAasy columns (Qiagen). The plasmids pR3N3-GFP-β-tubulin and pR3N3-histone H2b-RFP were a gift from K. Wassmann (CNRs, Paris, France; Touati et al., 2012). Mouse Cenp-V-Gfp was cloned into pOCC13 (Max Planck institute CBG, Dresden) plasmid and mRNA was transcribed and purified as above.

Oocytes arrested in prophase were injected using a FemtoJet microinjector (Eppendorf) with the target plasmid injected into the cytoplasmic pronucleus. For the microtubule-binding assays, taxol-stabilised Atto647N-labelled microtubules were bound to the anti-α-tubulin antibody for 2 h before being washed twice with M2. The cells were washed at RT in 0.1% Tween 20 in PBS 3 x 5 min and mounted in Vectashield (H-1000; Vector Laboratories) plus 1 μg/ml DAPI (1:5–6-diamidino-2-phenylindolod) dye for cytoplasmic imaging. Fixed oocytes were imaged with an inverted Zeiss Axio Imager Z2 microscope using a 63 x/1.4 NA oil objective. Brightness and contrast of the images were modified for presentations.

**Immuno-fluorescence and image acquisition.** For whole-mount immuno-fluorescence staining oocytes were cultured in M2 plus or minus 2.5 μM milrinone (Sigma) supplemented with 400 μM glucose oxidase (Serva Electrophoresis GmbH, Heidelberg, Germany) and 40 mM 2-deoxyribose (MP Biomedicals, Solon, OH) and punctured with a 27 gauge needle to release the oocytes. GV stage oocytes were collected with mouth pipettes (P200, Eppendorf) and prepared for immuno-fluorescence under paraffin oil (Sigma) at 37 °C. For analysis of GVBD and polar body extrusion oocytes were released into M2 medium and imaged every 30 min for up to 20 h on an inverted Nikon TE2000E microscope with a Plan APO 20 ×/0.75 NA objective, a CoolSNAP HQ camera (Photometrics), standard filter sets, and with an environmental chamber maintained at 37 °C. Z-series optical stacks of 80 μm were imaged with slices taken every 7 μm. All images were acquired using the Nikon NIS-Elements ND2 software and analyzed with ImageJ. Where indicated, reversine was added at 6 μM after GVBD at a final concentration of 300 nM. Because it is soluble in oil, reversine was also added to the oil covering the media drops.

**Generation of Cenp-V knockout mouse strain.** To generate a germ cell specific CENP-V deficiency, a Cenp-V allele was obtained through FLP recombinase FRT64--exon 3 of the gene is flanked by loxP sites to be recombined by Cre recombinase. The clones (Cenp-VloxP1/EUCOMM(Eapa)) received from EUCOMM, the relevant strain information can be found here: https://www.mousespotphenotype.org/data GENES/MGI:19203889/Order. An insertion cassette had to be deleted first by using FLP recombinase, which recombines the FRT sites. We have obtained highly (90 %) chimeric mice and have bred them with C57BL/6 mice. Germline transmission was confirmed by DNA sequencing. We used the following strains, all of which have been published and have been used in germ cells: (1) Vasa-Cre, which expresses Cre in late spermatogonia in males and at entry into meiosis in females; (2) GDF9-Cre, which expresses Cre in females at day 2 after birth, and (3) SPO11-Cre, where Cre is expressed in male and female early prophase.

**Oocyte chromosome spreads.** Oocytes chromosome spreads were prepared according to an adapted method from Hodges and Hunt, 2002. The zona pellucida of oocytes arrested at metaphases I was removed using acid treatment. Tyrode’s solution, (T1788; Sigma), and oocytes were transferred onto one well of a 10-well glass slide into 15 μl of fixation solution (1% PFA, 0.1% Triton X-100, 1 mM DTT; pH 9.2). After drying overnight at room temperature, slides were washed 3 x 5 min in PBS and stained immediately.

**Immunofluorescence and image acquisition.** For whole-mount immunofluorescence staining oocytes were cultured in M2 plus or minus 2.5 μM milrinone and collected at the relevant time points. The zona pellucida was removed using tyrode’s solution. After a 30 min recovery period, the oocytes were fixed in 2% formaldehyde and 0.1% Triton X-100 in M2 media for 10 min. Cells were washed once in blocking buffer (3-5 % bovine serum albumin (BSA) and 0.1 % Triton X-100 in phosphate-buffered saline (PBS)), before incubation in blocking buffer for 30 min at room temperature. The cells were transferred into the primary antibody solution (3% BSA and 0.1% Triton X-100) and incubated at 4 °C overnight. Primary antibodies were used as follows: rabbit anti-CENP-V (HPA042616; Sigma) at 1/100, rabbit anti-CENP-A (Homemade) at 1/250, rat anti-α-tubulin (MCA78G; Bio-Rad) at 1/5000, mouse anti-SYCP3 (clone 60C10) a gift from Dr. C. Heyting at Bio-Rad) at 1/5000, mouse anti-CENP-A (sc-11277; Santa Cruz) at 1/100. Secondary antibodies were used as follows: anti-rabbit Alexa Fluor 488 (A21042; Invitrogen) at 1/500, anti-mouse Alexa Fluor 647 (715-605-151; Jackson Immuno Research) at 1/500, anti-α-tubulin Alexa Fluor 488 (A21042; Invitrogen) at 1/500, anti-sheep Alexa Fluor 568 (A-20199; Invitrogen) at 1/500, anti-goat Alexa Fluor 488 (ab150129; abcam). The cells were washed at RT in 0.1% Tween 20 in PBS 3 x 5 min and mounted in Vectashield (H-1000; Vector Laboratories) plus 1 μg/ml DAPI (1:5–6-diamidino-2-phenylindolod) dye for cytoplasmic imaging. Fixed oocytes were imaged with an inverted Zeiss Axio Imager Z2 microscope using a 63 x/1.4 NA oil objective. Brightness and contrast of the images were modified for presentations.

**In vitro transfection, microinjection and oocyte live imaging.** β-tubulin-Gfp, histone H2b-Rfp, and Cenp-V-Gfp mRNAs were synthesized with the T3 or T7 Message mMachine Kit (Ambion) according to the manufacturer’s instructions and purified using RNAasy columns (Qiagen). The plasmids pR3N3-GFP-β-tubulin and pR3N3-histone H2b-RFP were a gift from K. Wassmann (CNRs, Paris, France; Touati et al., 2012). Mouse Cenp-V-Gfp was cloned into pOCC13 (Max Planck institute CBG, Dresden) plasmid and mRNA was transcribed and purified as above.

Oocytes arrested in prophase were injected using a FemtoJet microinjector (Eppendorf) with the target plasmid injected into the cytoplasmic pronucleus. For the microtubule-binding assays, taxol-stabilised Atto647N-labelled microtubules were bound to the anti-α-tubulin antibody for 2 h before being washed twice with M2. The cells were washed at RT in 0.1% Tween 20 in PBS 3 x 5 min and mounted in Vectashield (H-1000; Vector Laboratories) plus 1 μg/ml DAPI (1:5–6-diamidino-2-phenylindolod) dye for cytoplasmic imaging. Fixed oocytes were imaged with an inverted Zeiss Axio Imager Z2 microscope using a 63 x/1.4 NA oil objective. Brightness and contrast of the images were modified for presentations.
Cold treatment and Airy scan imaging. Microtubules were stabilized by fixation in 1.9% formaldehyde in BRB80 buffer (80 mM PIPES, 1 mM MgCl2, 1 mM EGTA, pH 6.8) after 5 min cold treatment in 80 mM PIPES, 1 mM MgCl2, pH 7.4, as described in 26. To obtain oocytes at metaphase II, cells were fixed after 6-8 h after the first polar body extrusion as 29. Images with kinetochore-MT attachments were collected with an Airyscan module using the 63x/1.4 NA objective. The z plane distance was 145 nm. The spindle axis was placed through the centre, and the spindle equator plane was perpendicular to the spindle axis and crossed it at the centre. A central region in the equatorial plane with a thickness of 100 pixels was used to quantify the fluorescence of microtubule forming K-fibers. Any chromosome entirely outside the region of the equatorial plate was considered misaligned. DNA was stained by Hoechst 33258 and centromeres were considered the strongest stained region in the chromosome. Two parallel lines to the equatorial plane were drawn into the poles to measure the spindle pole width. Images were processed by ZEN blue software with the Airyscan processing module and Fiji24.

Statistics and reproducibility. The data processing, statistical analysis, and plotting was performed in Fiji25, MatLab (Biplane), Excel (2015 Microsoft), and GraphPad Prism (GraphPad Software, Inc.). Error bars indicate means ± standard deviation (SD). Sample sizes and statistical tests are indicated in the figure’s legends. All experiments were performed a minimum of three times.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The data generated in this study has been deposed in the BioStudies database under accession code “S-BSS7699”. A Source Data file is also provided. Accession to the nucleotide sequence of the floxed Cenp-v locus is available at https://www.eumcrt.org/search?q=Cenp-v&h=Go and http://www.informatics.jax.org/alleles/MLG445304. All other relevant supporting data of the key findings of this study are available within the article and its Supplementary Information files or from the corresponding author upon reasonable request. Source data are provided with this paper.

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Author contributions

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The authors declare no competing interests.

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