During fertilization, an egg and a sperm fuse to form a new embryo. Eggs develop from oocytes in a process called meiosis. Meiosis in human oocytes is highly error-prone \(^1,2\), and defective eggs are the leading cause of pregnancy loss and several genetic disorders such as Down’s syndrome \(^3\). Which genes safeguard accurate progression through meiosis is largely unclear. Here we develop high-content phenotypic screening methods for the systematic identification of mammalian meiotic genes. We targeted 774 genes by RNA interference within follicle-enclosed mouse oocytes to block protein expression from an early stage of oocyte development onwards. We then analysed the function of several genes simultaneously by high-resolution imaging of chromosomes and microtubules in live oocytes and scored each oocyte quantitatively for 50 phenotypes, generating a comprehensive resource of meiotic gene function. The screen generated an unprecedented annotated data set of meiotic progression in 2,241 mammalian oocytes, which allowed us to analyse systematically which defects are linked to abnormal chromosome segregation during meiosis, identifying defects in spindle organization as risk factors. This study demonstrates how high-content screens can be performed in oocytes, and allows systematic studies of meiosis in mammals.

Meiosis is still much more poorly understood than mitosis, especially in mammals. Systematic screens have greatly increased our understanding of mitosis. However, high-content screens for mammalian meiotic genes have so far been precluded by various technical challenges. For instance, mammalian oocytes are only available in small numbers; genetic screens in mammals are slow; and RNA interference (RNAi) in oocytes is inefficient owing to large amounts of stored protein. Oocytes accumulate proteins while they grow within follicles in the ovary \(^4\). Thus, we established a protocol that allowed us to block protein expression by RNAi during follicle growth and subsequently to assess gene function by quantitative live imaging (Fig. 1a). Briefly, we microinjected short interfering RNAs (siRNAs) into small follicle-enclosed oocytes and grew the follicles in vitro, combining and modifying previous methods \(^7,8\). When the oocytes had reached their full size, we isolated and labelled them, and imaged meiosis live for around 18 h on confocal microscopes using automated imaging routines.

The oocytes grown in vitro resembled those grown in vivo: first, the efficiency of nuclear envelope breakdown (NEBD) and polar body extrusion, as well as the timing of meiotic progression, were similar (Fig. 1b–d and Extended Data Fig. 1d, e); second, their transcriptome was related (Extended Data Fig. 2a–c and Supplementary Table 1); third, they developed into blastocysts with similar efficiency upon fertilization (Extended Data Fig. 1f, g) \(^8\).

Follicle culture and microinjection are labour-intensive, precluding genome-wide screens. Instead, we preselected 774 target genes that were highly expressed in mouse oocytes, while excluding messenger RNAs (mRNAs) stored for embryo development. We took advantage
of two microarray data sets\textsuperscript{10,11}, which compare the expression profile of oocytes with the profiles of other cell types and preimplantation mouse embryos, respectively (Fig. 1f). Only genes that were significantly upregulated in oocytes in both data sets were selected for the mouse embryos, respectively (Fig. 1f). Only genes that were significantly upregulated in oocytes for the screen.

To achieve high throughput, we targeted 12 genes simultaneously (Fig. 1f), suggesting that it promotes NEBD by dephosphorylating the observed defects, siRNAs were microinjected again upon gene track down the genes that caused the phenotype of interest. To confirm the observed defects, siRNAs were microinjected again upon gene identification. In addition, specificity was confirmed by microinjection of individual siRNAs and rescue experiments as detailed below.

The screen identified several genes that control meiotic progression, including Dusp7, a poorly characterized dual-specificity phosphatase. More than 40% of Dusp7-depleted oocytes failed to undergo NEBD (Fig. 3a, b and Supplementary Video 1). In the remaining 60%, NEBD was significantly delayed (Fig. 3c). NEBD could be rescued by wild-type DUSP7 fused with enhanced green fluorescent protein (eGFP–DUSP7), but not by the catalytically inactive eGFP–DUSP7 C333S mutant (Fig. 3b), indicating that the phosphatase activity of DUSP7 is essential for NEBD. eGFP–DUSP7 was excluded from the nucleus (Fig. 3d), suggesting that it promotes NEBD by dephosphorylating cytoplasmic proteins. Together, these data identify Dusp7 as a phosphatase essential for NEBD in oocytes.
Spindle defects

**RESEARCH**

Figure 3 | Dusp7 and Mastl depletion phenotypes. a, Oocytes microinjected with control or Dusp7 siRNAs. Chromosomes in magenta. Quantification of phenotype in b, c. Scale bar, 10 μm. b, c, Efficiency (b) and timing of NEBD (c) in oocytes microinjected with Dusp7 siRNAs alone or together with mRNA encoding eGFP–DUSP7 or eGFP–DUSP7 C333S. d, Localization of DUSP7 during oocyte maturation. Live oocytes expressing DUSP7 fused with eGFP (green) and H2B fused with monomeric red fluorescent protein (mRFP; magenta, chromosomes). Scale bar, 10 μm. Representative for 36 oocytes from 5 experiments. e, Oocytes microinjected with control or Mastl siRNAs. Microtubules in green, chromosomes in magenta. Arrows highlight lagging chromosomes. Quantification of phenotypes in f–i. Scale bar, 10 μm. f–i, Oocytes microinjected with different Mastl siRNAs alone or together with mRNA encoding human eGFP–MASTL were scored for formation of pronuclei (f), lagging chromosomes (g), and efficiency (h) and timing of NEBD (i). Number of oocytes is given next to bars. P values were calculated with Fisher’s exact (b, g, h) or Student’s t-tests (c, i). Data from six (b, c), two (f) or five (g–i) independent experiments. The box plots in c and i show median (line), mean (small square), 5th, 95th (whiskers) and 25th and 75th percentile (boxes).

Figure 4 | Factors implicated in chromosome segregation errors. a, b, The efficiency (a) and timing (b) of progression into anaphase in control oocytes with aligned and misaligned chromosomes. Number of oocytes is given next to bars. P value was calculated with Fisher’s exact test. Data from 52 independent experiments. c, Defects significantly more likely to occur in oocytes with lagging chromosomes. Significance was calculated with Fisher’s exact test by comparing the prevalence of other defects in oocytes with and without lagging chromosomes, and is specified by asterisks next to arrows, with ***p < 0.0001; **p < 0.001; *p < 0.01; *p < 0.05. The circle area reflects the percentage of oocytes with lagging chromosomes in which each defect was observed.
Another gene essential for meiotic progression was Eif4enif1. Mutations in Eif4enif1 have recently been detected in a family with premature ovarian failure\(^1\), but the mechanism by which Eif4enif1 affects fertility is unclear. Our results show that Eif4enif1 is essential for NEBD and resumption of meiosis (Extended Data Fig. 6, a).

The screen also provided insights into causes of chromosome segregation errors in oocytes. Several genes were essential for accurate chromosome segregation, including the uncharacterized genes Fam46b and Fam46c (family with sequence similarity 46), Aspm\(^14\) (Extended Data Fig. 7 and Supplementary Video 2), Birc5 (Survivin)\(^15\) (Extended Data Figs 6), Ttk\(^16\) and Mastl (Fig. 3e, g). MASTL was also required to prevent exit from meiosis after anaphase I (Fig. 3e, f), but dispensable for meiotic resumption, progression into anaphase, chromosome condensation or cytokinesis (Fig. 3h, i and Extended Data Fig. 8), consistent with a recent study\(^17\).

The screen also allowed us to analyse on a global level how chromosome segregation errors arise in oocytes. With data from 2,241 oocytes, it generated the largest existing data set, to our knowledge, of meiosis in mammalian oocytes (Supplementary Table 2). Evaluation of the control data set identified progression into anaphase with misaligned chromosomes as a major contributor to chromosome segregation errors: misaligned chromosomes only delayed but did not prevent progression into anaphase (Fig. 4a, b). This is consistent with the model that the spindle assembly checkpoint in mammalian oocytes is less stringent than in mitosis\(^4,5\).

We were also able to analyse systematically which defects in the oocyte preceede chromosomes that lag behind during anaphase. This is of particular interest because lagging chromosomes can lead to inappropriate partitioning of chromosomes upon cytokinesis and are a major cause of aneuploidy\(^18,19\). We identified chromosome alignment, individualization and stretching as well as spindle defects as risk factors (Fig. 4c). A systematic representation of how different defects in oocytes were linked is shown in Extended Data Figs 9 and 10.

In summary, we have established an experimental system that now allows systematic studies of meiosis in mammals. The screening approach is scalable and could be adapted to investigate fertilization or embryo development. The follicle-based RNAi method will also be a powerful tool for individual gene studies, as it allows proteins with low turnover to be depleted in oocytes and pre-implantation embryos. The techniques presented in this study should thus facilitate a more rapid accumulation of knowledge about meiosis and early embryo development in mammals, which is crucial to improve methods for treating fertility problems in humans.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions All authors analysed data. S.P. microinjected the majority of siRNA mixes, and identified, validated and characterized most genes; M.P. microinjected siRNA mixes, compared the developmental capacity and expression profile of oocytes grown in vitro and in vivo, and identified, validated and characterized several genes; V.K. microinjected siRNA mixes and wrote software in OriginPro to quantify phenotypes; T.T. validated and characterized Dusp7; B.S. did all bioinformatics analyses; V.K. and M.S. developed and established the strategy of the screen; M.S. wrote the manuscript; S.P., M.P., T.T. and B.S. commented on and edited the manuscript; M.P. and M.S. prepared the revised manuscript; M.S. supervised the study.

Author Information RNA sequencing data have been deposited in Gene Expression Omnibus under accession number GSE68150. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.S. (mschuhr@mrc-lmb.cam.ac.uk).
METHODS

Preparation, microinjection and culture of follicles. All mice were maintained in a specific pathogen-free environment according to UK Home Office regulations. Ovaries were dissected from two to five 10- to 12-day-old (C57BL/× CBA) F1 females. To obtain individual follicles, the ovaries were incubated in modified MEM-2 (Gibco 12000-014) medium optimized for in vitro culture of follicles supplemented with 0.026 M NaHCO3 (Sigma), 5678 U 100 ml-1 penicillin G (Sigma) and 8265 U 100 ml-1 streptomycin (Sigma), 1× insulin/transferin/selenium solution (ITS; Sigma; stock was 100×), 1% fetal bovine serum (FBS; Gibco 16000044) and 0.01 μg ml-1 follicle stimulating hormone (FSH; National Hormone and Peptide Program, NDDK-oFSH-20) that was supplemented with 1 mg ml-1 collagenase (Roche) for about 30–40 min. During incubation with collagenase, the ovaries were pipetted up and down every 10 min to facilitate dissociation and then washed through several droplets of follicle culture medium without collagenase. The follicles where then randomly allocated into control siRNA and RNAi mix injection groups. Intact follicles were then loaded into a microinjection chamber prepared with two double stick tapes as spacer and microinjected as previously described2 in culture medium supplemented with HEPES (Sigma). Upon microinjection, follicles were cultured at 37 °C in 5% CO2 on membrane inserts in 6- or 12-well culture dishes filled with follicle culture medium (see above). For most experiments, collagen-coated inserts from Corning (Cornwell COL) and dialysis tubing (Amersham) were used. Follicles were coated with 10 μg cm-2 collagen solution type I from rat tail (Sigma), BD Matrigel Basement Membrane (BD Biosciences; thin coating method) as well as BD BioCoat filters were successfully used (Extended Data Fig. 1c). Medium surrounding the filter was replaced with fresh medium every 3–4 days. Oocytes were isolated from follicles after 10–11 days of in vitro culture. To this end, the oocytes were stripped with a small glass pipette and released into modified M2 medium that contained 10% FBS instead of BSA as well as 100 μg ml-1 Antibiotics and Penicillins in 50 ml of sperm source molecules. To identify significantly differentially expressed genes between oocytes grown in vitro and in vivo, we used a non-parametric method encoded in NOISeq. For this, we first filtered for low count or abundance using the ‘CPM’ low count filter of NOISeq. This yielded a reduction from the original 16,343 genes to 11,470 genes. Significant differential expression between oocytes grown in vivo and in vitro was determined using NOISeq with the following parameters: (1) ‘mm’, trimmed mean of log2, FPKM, normalization; (2) biological replicates data; and (3) probability of differential expression q being set to 0.8 or above and corrected for FDR. Our method yielded greater than 1–1 or 1–0 up- or downregulated genes, respectively in the in vitro group. This yielded 146 upregulated and 67 downregulated genes in oocytes grown in vitro. The vast majority of genes (11,110) were unchanged between the two conditions.

Quantitative real-time PCR. mRNA was extracted using an RNeasy Mini Kit (Qiagen) and cDNA was generated using a High Capacity RNA-to-cDNA Kit (Applied Biosystems). Real-time PCR was performed with the 7900 HT Real-Time Fast PCR System (Applied Biosystems) using SYBR Green. GAPDH mRNA was used for normalization.

RNA sequencing. Total RNA was isolated using NucleoSpin RNA XS (Macherey-Nagel) from oocytes grown in vitro after 10 days of follicle culture or from oocytes obtained directly from adult (C57BL/× CBA) F1 females (7–11 weeks old). A total of 50 oocytes with an intact nucleus per sample were used and three samples per group were collected. RNA was extracted using NucleoSpin RNA XS (Macherey-Nagel). A cDNA library was prepared using SMARTer UltraLow Input RNA for Sequencing (Clontech Laboratories) and the samples were processed by BGI Tech Solutions. The cDNA product was synthesized and amplified using a SMARTer PCR-cDNA Synthesis Kit (Clontech Laboratories) from the total RNA (10 ng) of each sample. The cDNA was fragmented by Covaris E210 and the median insert length was about 200 base pairs. The paired-end cDNA library was prepared in accordance with Illumina’s protocols with an insert size of 200 base pairs and sequenced for 100 base pairs by HiSeq2000 (Illumina).

Expression analysis. NOISeq. RNA-Seq based measurements of transcript abundances at the level of genes were represented by fragments per kilobase of transcript per million fragments mapped (FPKM). FPKM is conceptually similar to the reads per kilobase per million reads sequenced (RPKM) measure, but it is easily adaptable for sequencing data from one to higher numbers of reads from single source molecules. To identify significantly differentially expressed genes between oocytes grown in vitro and in vivo, we used a non-parametric method encoded in NOISeq. For this, we first filtered for low count or abundance using the ‘CPM’ low count filter of NOISeq. This yielded a reduction from the original 16,343 genes to 11,470 genes. Significant differential expression between oocytes grown in vivo and in vitro was determined using NOISeq with the following parameters: (1) ‘mm’, trimmed mean of log2, FPKM, normalization; (2) biological replicates data; and (3) probability of differential expression q being set to 0.8 or above and corrected for FDR. Our method yielded greater than 1–1 or 1–0 up- or downregulated genes, respectively in the in vitro group. This yielded 146 upregulated and 67 downregulated genes in oocytes grown in vitro. The vast majority of genes (11,110) were unchanged between the two conditions.

DESeq2. RNA-seq counts were considered with two ‘conditions’, namely in vitro and in vivo with three replicates. The standard protocol for DESeq2 differential expression analyses was followed with default settings. We deemed genes to be upregulated or downregulated if log2 values were greater than or equal to –1 or 1 for upregulated and downregulated genes respectively in the in vitro group, with a false discovery rate (FDR) or padj of DESeq2 less than 0.01 or 0.1. We considered a p-value below a low value of false discovery rate because of overall low expression levels for transcripts. Hence, we used a more stringent value for the false discovery rate. This yielded 282 upregulated and 163 downregulated genes in vitro.

Statistics. Mean, s.d. and statistical significance based on Student’s t-test or Fisher’s exact test (two-tailed) were calculated in Microsoft Excel, assuming normal distribution and similar variance. No statistical methods were used to pre-determine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment. All error bars show s.d. All box plots show median (line), mean (small square), 5th, 95th (whiskers) and 25th and 75th percentile (boxes). The z-scores were calculated according to the mean of the control group ± 1 SD as a mean of all controls of the RNAi screen, normalized to the s.d. of all controls. siRNA mixes were sorted according to their z-score. The dashed line in Fig. 2 and Extended Data Fig. 4 delineates mixes with a z-score higher than two s.d. above the average value of all controls.

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Data analysis. Phenotypes were evaluated manually by browsing the data in Zen (Zeiss). Defects and measurements (time points and spindle parameters) were then recorded on a homemade user interface in OriginPro 8.0 and processed in Microsoft Excel. Averages and statistical significance were calculated in Excel. The z-scores were calculated as the deviation of the mean of a single mix to the overall mean of all controls, normalized to the s.d. of all controls. Oocytes that died during imaging were not analysed and do not contribute to the data set.

For Fig. 4c, we analysed data from all 2,241 oocytes, because lagging chromosomes are not very common in control oocytes, but are likely to be triggered by various defects such as those induced by RNAi in the screen.

For the Jaccard index heatmap in Extended Data Fig. 9, RNAi screen phenotypes from both mix and control experiments were collected; wherever there were numerical values, they were converted appropriately into ‘yes’ and ‘no’ values based on the mean and s.d. of the distribution of numerical values. Further, ‘yes’ values were categorized into ‘+’ and ‘−’ groups based on whether a numerical entry was greater than mean + s.d. or smaller than mean – s.d. This information was converted to a network representation such that there were two types of node in the network oocytes and phenotypes (Extended Data Figure 9c). An edge was made between oocyte and phenotype if a given oocyte scored ‘yes’ for a given phenotype. This yielded a network that we termed phenotype–oocyte network, which included 5,203 edges (or associations) between 53 phenotypes and 1,504 oocytes. The distribution of the number of oocytes against the number of distinct phenotypes scored in them suggested that over 75% of oocytes, namely 1,195, have two or more phenotypes scored, suggesting that there were widespread multiple phenotypes scored in them. Hence, we sought to estimate the extent of co-occurring phenotypes across oocytes as a first step towards phenotype correlations. We calculated the Jaccard index between all possible pairs of phenotypes in the phenotype–oocyte network. This yielded a network that we termed phenotype–oocyte network, which we termed phenotype–oocyte network, which we termed phenotype–oocyte network, which we termed phenotype–oocyte network.

Oocytes that died during imaging were not analysed and do not contribute to the data set.

The above formula for the Jaccard index captures the fraction of co-occurrence of phenotypes i and j in oocytes over the total observed number of instances of phenotypes i or j. The numerator denotes the number of oocytes in which phenotypes i and j were observed, while the denominator indicates the total number of oocytes in which either phenotypes i or j have been observed. The values of a Jaccard index range between 0 and 1. Zero signifies poor co-occurrence while ‘1’ signifies high co-occurrence. We calculated the Jaccard index for all possible pairs of phenotypes in the phenotype–oocyte network. Out of a possible 1,378 (53C2), we could obtain 844 pairs that displayed a Jaccard index greater than zero. We then clustered the profile of the Jaccard index between phenotypes represented as a matrix or table. For this purpose, we used pheatmap (http://cran.r-project.org/web/packages/pheatmap/index.html) with ‘mean’ clustering and ‘Pearson correlation’ options. In this way, we obtained three major clusters of phenotype correlations.

Measurement of oocyte diameter, spindle length and spindle width. Oocyte diameter, spindle length and width in metaphase I and metaphase II were measured using the Measurement function in Zen (Zeiss). To measure the oocyte diameter accurately, measurements were always taken in the centre of the oocyte as determined by the maximum radius of the oocyte. Spindle length and width were only measured in oocytes in which the spindle was parallel to the confocal imaging plane.

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Extended Data Figure 1 | Efficiency of follicle growth and comparison of oocytes grown in vitro and in vivo. a, Follicles before (top panel) and after (bottom panel) in vitro culture. The perimeters of oocyte and granulosa cells are highlighted on the right. The follicle diameter increases from 103.4 ± 11.3 μm to 314.1 ± 104.0 μm during in vitro culture. This lies between the diameter of in vivo grown early antral (≈248 μm) and Graafian (≈424 μm) mouse follicles 26. The diameter of n follicles was measured before and after in vitro culture and is displayed as mean ± s.d. Measurements from three or two independent experiments for before and after culture, respectively. b, Diameter of oocytes grown in vivo or in vitro. Data from two and seven experiments, respectively. The box plot shows median (line), mean (small square), 5th, 95th (whiskers) and 25th and 75th percentile (boxes). c, Efficiency of follicle growth on different culture substrates. The numbers of independent experiments are 343, 56, 11 and 3 from left to right. The total number of follicles is specified above the bars. Error bars, s.d. d, Live oocyte expressing eGFP–MAP4 (green, microtubules) and H2B–mRFP (magenta, chromosomes). The characteristic time points of oocyte maturation that were determined for each oocyte in the screen (2,241 oocytes in total from 70 experiments) are listed above the representative images. Quantification of timing in e. Scale bar, 10 μm. e, The timing of bipolar spindle assembly, chromosome alignment during meiosis I, anaphase, polar body extrusion and chromosome alignment during meiosis II were quantified in oocytes obtained from 5-week-old (C57BL × CBA) F1 females or in oocytes from the same strain grown in vitro within follicles. Data from four independent experiments. Error bars, s.d. f, Transmitted light images of blastocysts derived from fertilized (C57BL × CBA) F1 oocytes grown in vitro within follicles (left) or in vivo (right). Scale bar, 20 μm. Quantifications in g. g, (C57BL × CBA) F1 oocytes grown in vitro within follicles or in vivo were denuded, matured in vitro and fertilized. The percentages of all oocytes (fertilized and unfertilized) that developed into two-cell embryos (two-cell from total) and two-cell embryos that developed into blastocysts (blastocyst from total) were quantified. Developmental rates are consistent with previous studies, in which in vitro matured denuded oocytes were fertilized 27,28; 179 oocytes grown in vivo and 180 oocytes grown in vitro were analysed in total. Data from three independent experiments for each group. Error bars, s.d. h, Transmitted light images of control oocytes and oocytes microinjected with an siRNA mix targeting Zp3 together with 11 other genes (RNAi Mix against Zp3) or an siRNA mix microinjected at the same time that targeted 12 other genes (RNAi mix against other genes). Highlighted region is magnified below. Scale bar, 10 μm. Quantification of phenotype in i. i, The presence of the zona pellucida was scored in oocytes microinjected with control siRNA (control), an siRNA mix targeting one of the three Zp genes (Zp1, Zp2 or Zp3) together with 11 other genes and an siRNA mix microinjected at the same time that targeted 12 different genes (RNAi mix against other genes). The number of analysed oocytes is given next to bars.
Extended Data Figure 2 | Transcriptome analysis of oocytes grown in vivo and in vitro. a–c, Transcriptome analysis of oocytes grown in vitro and in vivo. a, Differentially expressed genes in oocytes grown in vitro based on evaluation using NOISeq algorithm. Transcript abundances are reported in transcript FPKM. Only about 2% (213 out of 11,470) of genes were differentially expressed. b, Differentially expressed genes in oocytes cultured in vitro based on evaluation using DESeq2 algorithm. Only about 4% (445 genes out of 10,597) of genes were differentially expressed after applying filters in both b and c. The blue lines indicate genes with at least twofold change in expression. Red colour indicates differentially expressed genes with the denoted probability. For details, see Methods. c, The overlap between NOISeq and DESeq2 results, presented as Venn diagrams. There is at least over 80% overlap in genes in either upregulated or downregulated groups for both NOISeq and DESeq2. d, Qualitative network of phenotypes in oocytes microinjected with siRNA mixes. Blue nodes represent siRNA mixes, purple nodes represent phenotypes. Grey lines between mixes and phenotypes denote if at least one oocyte microinjected with a given mix displayed the phenotype. The clusters indicate a close relationship between a set of phenotypes and mixes. The clusters were obtained using ClusterViz (https://code.google.com/p/clusterviz-cytoscape/) of Cytoscape, which encodes the MCODE method to identify clusters of closed related nodes based on the topology of the network. The network contains six clusters identified by ClusterViz.
Extended Data Figure 3 | Description of defects scored in screen. 

**a.** Scheme illustrating the main categories of defects that were quantified in the screen.

**b.** Table listing the main categories of defects and their subcategories as well as a description of each defect.

**c.** Table listing the numerical values that were measured in the screen and a description of each numerical value.
Extended Data Figure 4 | Defects during meiosis II in siRNA-treated oocytes. a, d, g. The frequency of cytokinetic defects (a), spindle defects in metaphase II (d) and chromosome defects in metaphase II (g) were scored in siRNA-treated oocytes. The absolute number of oocytes with each defect is given next to bars. Data from 70 independent experiments. Corresponding control data are shown in Extended Data Fig. 5.

b, e, h. Examples of defects in live oocytes. Chromosomes (magenta) were labelled with H2B–mRFP, microtubules (green) with eGFP–α-tubulin. Quantifications in a, d, g. Scale bars, 10 μm.

c, f, i. The z-scores were calculated as the deviation of the mean of a single siRNA mix to the mean of all controls of the RNAi screen, normalized to the s.d. of all controls. siRNA mixes were sorted according to their z-score. The dashed line delineates mixes with a z-score higher than two s.d. above the average value of all controls.

j, List of genes that were tracked down to the individual gene level in the RNAi screen. Note that defects caused by depletion of some proteins such as Zfp420 or Uhrf1 may reflect the function of more proximal genes under the control of these proteins. We were able to allocate 16 out of 20 tested defects to individual genes. Defects that could not be tracked down to individual gene level are shown as grey bars ending after the second or third round.
Extended Data Figure 5 | Frequency of meiosis I and meiosis II defects in oocytes treated with control siRNAs. a–g. The frequency of scored general morphological defects (a), spindle defects in meiosis I (b), chromosome defects in meiosis I (c), defects in anaphase I (d), defects during cytokinesis (e), spindle defects in meiosis II (f) and chromosome defects in meiosis II (g) were scored in oocytes microinjected with control siRNAs. The absolute number of oocytes with each defect is given next to bars.
Extended Data Figure 6 | Eif4enif1 is required for release from prophase arrest and Birc5 for spindle integrity. a, Live oocytes microinjected with control siRNA (control) or siRNAs targeting Eif4enif1 (Eif4enif1 RNAi) expressing eGFP–α-tubulin (green, microtubules) and H2B–mRFP (magenta, chromosomes) merged with differential interference contrast (DIC) image. Region of spindle and chromosomes is magnified without DIC below. Quantification of phenotype in b. Scale bar, 10 μm. b, Live oocytes microinjected with control siRNA or Eif4enif1 siRNAs were monitored by long-term time-lapse microscopy as shown in a and the efficiency of NEBD was scored. The number of analysed oocytes is specified next to bars. The P value was calculated with Fisher's exact test. Data from a total of three experiments. c, Live oocytes microinjected with control siRNA (control) or siRNAs targeting Birc5 (Birc5 RNAi) expressing eGFP–α-tubulin (green, microtubules) and H2B–mRFP (magenta, chromosomes) merged with DIC. Region of spindle and chromosomes is magnified without DIC below. Quantification of phenotypes in d–g. Scale bar, 10 μm. d, Live oocytes microinjected with control siRNA (control), a mix of three different Birc5 siRNAs (siRNA 1–3) or two Birc5 siRNAs individually (siRNA 1, 2) were scored for temporary or permanent disintegration of the meiotic spindle. The number of analysed oocytes is specified next to bars. The P value was calculated with Fisher’s exact test comparing control and all Birc5 siRNA microinjected oocytes from five experiments. e–g, Live oocytes microinjected with control siRNA or Birc5 siRNAs were monitored by long-term time-lapse microscopy as shown in c and the efficiency of NEBD (e), the presence or absence of misaligned chromosomes (f) as well as the efficiency of chromosome segregation (g) were scored. The number of analysed oocytes is specified next to bars. P values were calculated with Fisher’s exact test. Data (d–g) from five independent experiments.
Extended Data Figure 7 | Aspm function in mouse oocytes. a, Oocytes microinjected with siRNAs targeting Aspm or injected with control siRNA. Microtubules in green, chromosomes in magenta. Arrows highlight lagging chromosomes. Quantification of phenotypes in b–g. Scale bar, 10 μm. b, c, Lagging (b) or misaligned chromosomes (c) in oocytes microinjected with different Aspm siRNAs. d–g, Live oocytes microinjected with control siRNA (control) or Aspm siRNAs (Aspm RNAi) were monitored by long-term time-lapse microscopy as shown in a and scored for progression through anaphase (d), time of anaphase onset (e), polar body extrusion (f) and spindle length (g). The number of analysed oocytes is specified next to bars. The $P$ value was calculated with Fisher’s exact test (b, c, d, f) or Student’s $t$-test (e, g) comparing control and all Aspm siRNA microinjected oocytes. The box plots in e and g show median (line), mean (small square), 5th, 95th (whiskers) and 25th and 75th percentile (boxes). Data from four independent experiments.
Extended Data Figure 8 | Mastl is required for metaphase II arrest and accurate chromosome segregation, but is dispensable for cytokinesis and chromosome condensation in mouse oocytes. a, Live oocytes microinjected with control siRNA (control) or siRNAs targeting Mastl (Mastl RNAi) expressing eGFP–Lamin B1 (green, nuclear lamina) and H2B–mRFP (magenta, chromosomes) merged with DIC. Representative of 30 control and 16 Mastl RNAi oocytes. Scale bar, 10 μm. b, c, Live oocytes microinjected with a mix of three different Mastl siRNAs expressing human Greatwall fused with eGFP (green) and H2B–mRFP (magenta, chromosomes) merged with DIC. eGFP–Greatwall localized to the nucleus and was released into the cytoplasm shortly before NEBD, consistent with previous studies in mitotic cells. Representative of 23 oocytes. Quantification in Fig. 3f. d–g, Live oocytes microinjected with control siRNA or Mastl siRNAs were monitored by long-term time-lapse microscopy and scored for anaphase progression (d), time of anaphase onset (e), successful formation or retraction of a polar body upon anaphase (f) and the prolonged presence of a midbody upon cytokinesis (g). The number of analysed oocytes is specified next to bars. Data from five independent experiments. h, Maximum z-projection (left) and three-dimensional reconstruction (right) of chromosomes (Hoechst) in fixed mouse oocytes microinjected with control siRNAs or siRNAs targeting Mastl were generated in Imaris. Quantification in i, l. The chromosome volume was quantified in mouse oocytes microinjected with control siRNAs or siRNAs targeting Mastl as shown in h in Imaris. The number of analysed oocytes is specified next to bars. Data from two independent experiments. j, Mastl mRNA levels in control oocytes and oocytes microinjected with Mastl siRNAs were quantified by real-time PCR. Mean values from two independent experiments. P values were calculated with Fisher’s exact test (d, f, g) or Student’s t-test (e, i). The box plots in e and j show median (line), mean (small square), 5th, 95th (whiskers) and 25th and 75th percentile (boxes).
Extended Data Figure 9 | Systematic analysis of phenotype correlations in mouse oocytes. a, b, Heatmap representation of clusters of phenotypes generated based on Jaccard indices between them. Jaccard indices, range between 0 and 1, were calculated as described in Methods and Extended Data Fig. 10. Jaccard indices calculated from control oocytes (a) and RNAi-treated oocytes (b) are shown. The ‘red’ and ‘blue’ respectively correspond to high and low Jaccard indices as indicated by the legend. Clusters of phenotypes were generated using Pheatmap with ‘Pearson correlation’ values and ‘average’ clustering input parameters.
Extended Data Figure 10 | Network of phenotypes and calculation of Jaccard indices. a, Network of phenotype to oocytes was converted into a phenotype–phenotype network based on number of oocytes that display two phenotypes in question. The network consists of 53 phenotypes and 867 connections between them. The nodes in the network denote phenotypes and edges denote shared oocytes. This is a qualitative network and does not consider the strength of connection, edge weight or number of oocytes in which a given pair of phenotypes co-occurs. Nodes of identical colours denote a cluster (a group of related phenotypes based on topological properties of the network). Phenotypes that are not part of any cluster are in the centre and indicated by squares (white). Related clusters (if they share phenotypes) are marked by dashed circles considered as ‘superclusters’. Clusters were identified by the NeMo method in Cytoscape. Network clusters are purely based on topological properties and are in agreement with the clusters in the heatmap constructed using quantitative measures of Jaccard indices (Extended Data Fig. 9a): for example, two superclusters, top left and top right respectively, correspond to heatmap clusters at the top left and middle of Extended Data Fig. 9a. b–d, Overview of computational approach with schematics to decipher phenotype clusters. O, M and N correspond to oocyte i, mix i and numerical value of phenotype i, respectively. b, Conversion of yes, no and numerical data depicts the way we converted a combination of ‘yes’, ‘no’ and numerical data (denoted by N1, N2, N3 and N4) of phenotypes across oocytes into purely ‘yes’ and ‘no’ groups with the ‘yes’ group further classified as ‘yes’+ and ‘yes’−. c, Reconstruction of the phenotype–oocyte network: we reconstructed a phenotype–oocyte network from the above data of ‘yes’ and ‘no’ values by considering only the ‘yes’ group. A nonlinear decay relationship between the number of phenotypes and number of oocytes in the network is displayed as represented by two plots. Details of the plots suggest a median value of 2 for phenotypes. d, Network transformation and calculation of Jaccard index matrix illustrate our network transformation strategy from a phenotype–oocyte network to a phenotype–phenotype network and the simultaneous estimation of Jaccard indices between phenotypes. The matrix of Jaccard indices between phenotypes was clustered using the pheatmap software in the R package with the ‘Pearson correlation’ parameter and the ‘average’ clustering method.